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THE UNIVERSITY OF ALBERTA

Production, morphology, and cytology of induced polyploids of *Elymus canadensis* L.

by

Cheol Ho Park

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF Doctor of Philosophy
IN
Plant Breeding

Department of Plant Science

EDMONTON, ALBERTA
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K.H. Asay
External Examiner

Date: 11 Dec 1989

DEDICATION

To my family

ABSTRACT

To examine germplasm potential for forage production, different ploidy levels of *Elymus canadensis* were induced and their genomic constitutions were modified. The chromosome numbers of hybrids derived from crosses between *Elymus canadensis* (SSHH) and *Psathyrostachys juncea* (NN) and *Secale cereale* (RR) were doubled to produce allopolyploids. Somatic tissue culture and subsequent interploidy hybridization were applied to produce autoallopolyploids of *E. canadensis*. As a result, triploids (SHN and SHR), tetraploids (SHRR), pentaploids (SSHHH or SSSHH), hexaploids (SSHHNN, SSHHRR, and SSSHHH), octoploids (SSSSHHHH), and aneuploids, including tetrasomics and trisomics were produced and examined morphologically and cytologically. Somaclones of *E. canadensis* in which chromosome numbers were not changed were also evaluated morphologically and cytologically in relation to forage improvement. The results are summarized as follows:

1. Sterile allotriploid hybrids with genomes from *E. canadensis*, *P. juncea*, and *S. cereale* were more productive than the parents, accounting for forage yield increases of 8% in SHN hybrids and 64% in SHR hybrids, respectively, compared to the higher-yielding parent, *E. canadensis*, grown under greenhouse conditions.
2. Allohexaploids with the genome constitutions SSHHNN and SSHHRR showed increased intragenomic bivalent formation and partial fertility. Allohexaploids indicated remote phylogenetic relationships among S, H, and N and among S, H, and R genomes.

3. Induced autoallooctoploids (SSSSHHHH) behaved cytologically like diploids and were fertile. An octoploid regenerant yielded 92% octoploid progeny in the first selfed generation.

4. Vigorous hexaploids with the genome constitutions SSSHHH were derived from intercrosses and backcrosses of octoploids with tetraploids. These hexaploids were found to be useful agronomically and yielded primary aneuploid stocks such as tetrasomics and trisomics for studies of genetics and genome relationships and an understanding of the nature of the genome.

5. Tissue culture was effective in elevating the ploidy level of *E. canadensis* and also contributed to small changes in chromosome structure. Callus cultures of hybrid embryos and subsequent plant regeneration was used as an alternative to embryo rescue and seemed to overcome hybrid necrosis in the SHR hybrid regenerant.

6. None of the intergeneric hybrids and somaclones of *E. canadensis* were suitable for direct use as forage germplasm because of poor plant vigor, low seed set, and cytological instability in advanced generations. Some of them would be useful for the production of alien chromosome addition or substitution lines and as aneuploid stocks for genetic studies.

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1. INTRODUCTION

1.1. Forage grass breeding

Native grasses may be used to seed abandoned farmland, severely eroded sites, droughty soils, rangelands, and other areas where cultivated grasses often fail (Schwendiman and Hawk 1973). The Canadian native grass species are also vast sources of potentially valuable genetic variability which confer resistance to local disease and insect pests and enable the plants to withstand stresses caused by drought as well as high summer and low winter temperatures. On the other hand, cultivated forage species, almost all of which have been introduced into Canada from Europe and Asia, give high yields of good quality forage and can withstand repeated defoliation.

Since 1930, efforts have been made to develop new cultivars from existing native and introduced cultivated forage grasses and to combine the favorable characteristics of the native and introduced species. A number of improved cultivars of native and introduced grasses have been released from public breeding programs and have added significantly to the germplasm resources available (Schwendiman and Hawk 1973; Asay and Knowles 1985a).

Mass and recurrent selection are widely used forage-breeding methods. They are applied to genetically diverse materials which are frequently obtained from existing cultivars, from material collected in the field, or from crosses (Poehlman 1978; Walton 1983). Intraspecific and interspecific hybridization, common practices for self-pollinated crops, has also been utilized. Interspecific crosses offer opportunities to obtain genotypes not possible with intraspecific hybridization, and to extend the range of genetic variability beyond that of a single species (Hadley and Openshaw 1980; Asay and Knowles 1985b). Briggs and Knowles (1967) have listed four reasons for making interspecific and intergeneric hybrids: (1) to transfer one or a few genes from one species to another, (2) to achieve new character expression not found in either parent, (3) to produce new allopolyploid species, and (4) to determine the relationship of one species to another.

It is generally believed that polyploidy is a useful method for breeding forages (Dewey 1979a). Because many species of forage grasses are already polyploids and do not tolerate further increases in chromosome number, attention in breeding autopolyploids is generally given to diploid species with low chromosome numbers (Dewey 1979a). The production of allopolyploids (amphiploids) offers opportunities for combining the desirable characteristics of two related species. Most success is attained by combining two species at allotetraploid and allohexaploid levels (Dewey 1984a).

Significant progress has been made toward improvement of annual Triticeae cereal crops by using new techniques such as in vitro culture (Larkin and Scowcroft 1981; Larkin et al. 1984) and recombinant DNA technology, thus opening up possibilities to combine new characteristics with precision (Lorz et al. 1988). Given improved callus formation and plant regeneration in Graminaceous species, a number of forage grasses have been cultured in vitro (Vasil 1982). Some show genetic stability leading to micropropagation but many are genetically and cytologically unstable with potential of somaclonal variation to forage breeding. (Vasil 1987; Larkin 1987).

Objectives in breeding forage crops vary with the species, the region of production, and utilization of the crop for hay, pasture, turf, or other purposes. Ultimately, it is necessary to study each species individually, to identify the conditions that are limiting production and quality in the area where the breeder is working, and to determine whether heritable improvement may be made that will reduce the losses from those causes (Poehlman 1978).

Canada wildrye (*Elymus canadensis* L.) is a large, coarse, short-lived perennial, self-fertilizing bunchgrass, widely distributed in the U.S. and Canada (Bowden 1964). It is a cool-season grass but begins growth later in the spring, and its growth lasts longer into the summer than that of crested wheatgrass and smooth bromegrass. Its palatability is fairly good and it produces the best hay when cut at the boot stage (Walton 1983). Seedlings are vigorous, establish quickly and are not highly competitive with other grasses in mixtures (Schwendiman and Hawk 1973). The species is known to tolerate many diseases and drought. Therefore, this species is important in forage production, range value, soil conservation, and as a genetic reservoir for the improvement of cereals. For these reasons, many researchers have studied Canada wildrye (Mujeeb-kazi and Rodriguez 1982; Mujeeb-kazi and Bernard 1982, 1985; Dewey 1984b (review); Hang and Franckowiak 1984; Yen and Liu 1987).

1.2. Genomic systems for classifying the perennial Triticeae

This study follows the genomic system of classification of the Triticeae in which Dewey (1984b) recognized *Agropyron*, *Pseudoroegneria*, *Psathyrostachys*, *Critesion*, *Thinopyrum*, *Elytrigia*, *Elymus*, *Leymus*, and *Pascopyrum* as the major perennial genera of the Triticeae tribe on the basis of their gross morphology and cytogenetic features. The type species, genomic formulae, approximate number of species, and chromosome number of the perennial Triticeae grasses are listed in Table 1.1 (Dewey, 1984b). Traditional nomenclature of the Triticeae species refered in this study is listed in the Appendix.

Table 1.1. The perennial genera of the members of the tribe Triticeae as determined by their genome content.

Genus	Type species	Genomes	No. of species	No. of chromosomes(2n)
Agropyron	*A. cristatum*	P	5	14, 28, 42
Pseudoroegneria	*P. spicata*	S	15	14, 28
Psathyrostachys	*P. lanuginosa*	N	5	14
Critesion	*C. jubatum*	H	30	14, 28, 42
Thinopyrum	*T. junceum*	J	20	14, 28, 42, 56, 70
Elytrigia	*E. repens*	SX	5	42, 56
Elymus	*E. sibiricus*	SHY	150	28, 42, 56
Leymus	*L. arenarius*	JN	30	28, 42, 56, 70, 84
Pascopyrum	*P. smithii*	SHJN	1	56

1.3. Genome constitution of native *Elymus*

Elymus is the most widely distributed polyploid genus in the Triticeae. These species occur naturally in Europe, Asia, North America, South America, New Zealand, and Australia (Dewey, 1984b). About 75% of the species are allotetraploids. Most allotetraploid *Elymus* species have the genome constitution SSHH, with the S genome derived from *Pseudoroegneria* (SS) and the H genome derived from *Critesion* (=*Hordeum* HH) (Stebbins and Snyder 1956; Dewey 1971; Dewey, 1974b; Wang and Hsiao 1986). Many *Elymus* species are from Europe (*E. caninus, E. alaskanus*), Central Asia (*E. fibrosus, E. mutabilis*), North America (*E. canadensis, E. trachycaulus, E. lanceolatus, E. glaucus*), and South America (*E. tilcarensis, E. agropyroides*) but each has its own variation of the S and H genomes (Dewey, 1984b). *E. dentatus* ssp. *ugamicus* (Dewey 1980a), *E. ciliaris* (Dewey 1984b), *E. panormitanus* (Jensen and Hatch 1988), *E. strictus, E. gemelinii* (Jensen and Hatch 1989), and *E. alboinii* (Jensen 1989) have SSYY genomes. The source of the Y genome is unknown. *Elymus drobovii* (Dewey 1980b) and *E. tsukushiense* (Sakamoto and Muramatsu 1966b) are allohexaploids with a genome formula of SSHHYY. *E. alatavicus* and *E. batalinii* contain an S and probably a Y genome plus an unknown genome, X (Jensen et al. 1986). In the genus *Elymus*, segmental autoallohexaploids (SSS'S'HH or SSHHH'H') are relatively common accounting for 21% of the total *Elymus* species(Dewey, 1984b) and an allohexaploid (SSSHHH) has been found in natural tetraploid populations of *Elymus lanceolatus* by Sadasivaiah and Weijer (1981). Octoploids (SSS'S'HHH'H') are rare, accounting for less than 3% of the population (Bowden 1964; Dewey, 1984b). That natural octoploids occur uncommonly indicates that octoploidy is beyond the optimum chromosome level for *Elymus* species (Dewy 1984b).

1.4. Genome manipulation in *Elymus*

1.4.1. Induced polyploidy

The discovery of colchicine enabled the creation of polyploid forms for many species (Eigsti and Dustin 1955). In the tribe Triticeae, induction of autotetraploids from diploid species has great plant breeding potential in *Agropyron*, *Pseudoroegneria*, and *Psathyrostachys*. Segmental allopolyploids and genomic allopolyploids are advantageous for the polyploid species of most genera (Dewey 1979a, 1984a). In contrast to other diploid species, the doubling of chromosome numbers of *Elymus* has not received much attention from plant breeders because doubling of chromosome numbers at the octoploid level causes a reduction in fertility and vigor (Asay and Dewey 1976; Dewey 1979a). However, polyploidy in *Elymus* has been promising for forage improvement at the hexaploid level since fertility, dry matter yield, and cytological stability may all be improved (Dewey 1984a). There are several types of induced polyploids in *Elymus*. These include:

(1) segmental autoallohexaploids of the type $SSS^{\prime}S^{\prime}HH$ or $SSHHH^{\prime}H^{\prime}$ produced by doubling the chromosome number of F_1 hybrids from crosses between tetraploid *Elymus* and diploid *Pseudoroegneria* or *Critesion* (Dewey 1967a,1974a; Asay et al. 1988).

(2) induced hexaploids (SSSHHH) from a cross between the colchicine-induced octoploid (SSSSHHHH) and tetraploid (SSHH) of *Elymus trachycaulus* (Aung and Walton 1987a),

(3) interspecific and interploidy hexaploid hybrids [SSS'HHH' from a cross, 8X (*Elymus trachycaulus*, SSSSHHHH) x 4X (*Elymus canadensis*, S'S'H'H')] (Aung and Walton 1989),

(4) interspecific hybrids (SS'HH'YY') from crosses between natural allohexaploids with genome constitutions SSHHYY and S'S'H'H'Y'Y' genomes (Sakamoto and Muramatsu 1966b),

(5) autoallooctoploids (SSSSHHHH) obtained by doubling the chromosome numbers of SSHH allotetraploids (Napier and Walton 1983; Aung and Walton 1987a), and

(6) autoallooctoploids (SSS'S'HHH'H') obtained by doubling the chromosome numbers of F_1 hybrids derived from crosses between different tetraploid *Elymus* species (Dewey 1968d; Dewey 1977b; Kumar and Walton 1989).

1.4.2. Interspecific hybridization

Approximately 40 interspecific hybrids have been derived from crosses between allotetraploid *Elymus* species. They have the genome formula SSHH or SSYY (Stebbins et al. 1946; Stebbins and Snyder 1956; Brown and Pratt 1960; Boyle 1963; Dewey 1965, 1966b, 1967a,b,c, 1968a,b,c,d, 1969a,b, 1974a, 1977a,b, 1979b, 1981; Sakamoto and Muramatsu 1966a; Gupta et al. 1988; Jensen and Hatch 1988, 1989). A few of these are found in nature (Dewey 1963; Collins 1966). Cross compatibility between *Elymus* species with the same genomes varies from cross to cross (0 to 71% of the total pollinated florets). Most of the tetraploid hybrids are morphologically intermediate between their parents and show low rates of pollen stainability, and complete sterility. The cause of sterility might be chromosomal, due to heterozygosity of small structural differences between closely related species with the same genomes (Dewey 1966b; Sakamoto and Muramatsu 1966a; Asay and Dewey 1976). Cryptic structural hybridity or genic causes have also contributed to sterility (Dewey 1966b; Dewey 1968d; Kumar and Walton 1989). The interspecific hybrids predominately form bivalents in the majority of meiocytes at metaphase I (9.26 to 13.97 bivalents per cell) indicating relatedness and allosyndesis between the parental genomes. Gross structural differences give rise to multivalent associations and chromosome bridges, leading to partial sterility (Dewey 1966b, 1977b; Sakamoto and Muramatsu 1966a).

Elymus canadensis hybridizes naturally with its relatives such as *E. virginicus*, *E. weigandii*, and *E. villosus* (Bowden 1964). *Elymus canadensis* hybridizes artificially and shows genomic relatedness with *E. glaucus* (Dewey 1965), *E. elymoides* (Dewey 1967a), *E. lanceolatus* (Dewey 1967c), *E. trachycaulus* (Dewey 1968d; Aung and Walton 1989), *E. trachycaulus* ssp. *subsecundus* (Dewey 1966b, 1977b), *E. semicostatus* (Dewey 1968d), *E. albicans* (Dewey 1969a), *E. sibiricus* (Dewey 1974a), *E. strictus* (Jensen and Hatch 1989), and *E. arizonicus* (Jensen et al. 1989). However, a few interspecific hybrids, *E. canadensis* x *E. semicostatus* (Dewey 1968d) *E. strictus* x *E. canadensis*, *E. strictus* x *E. lanceolatus* (Jensen and Hatch 1989), and *E. ciliaris* x *E. trachycaulus* (Sakamoto and Muramatsu 1966a) exhibit genomic differentiation as evidenced by a low frequences of bivalents

(5.31 to 5.81 bivalents per cell). Chromosome pairing in these hybrids demonstrates that *E. semicostatus*, *E. strictus* , and *E. ciliaris* contain the S and Y genomes.

Most of the hybrids do not show potential as forage grasses because they are sterile and lack vigor and so do not merit further consideration from plant breeders. Four interspecific hybrids (*E. canadensis* x *E. albicans*, *E. canadensis* x *E. caninus*, *E. mutabilis* x *E. caninus*, and *E. caninus* x *E. sibiricus*), having a genome constitution of SS'HH', are more productive in forage yield but all of them have low fertility or complete sterility (Dewey 1968d, 1969a, 1974a, 1979b). Sterility provides an effective barrier to introgression between the parental species. Interspecific hybrids between *E. dentatus* ssp. *ugamicus* (genomes SSYY) and *E. seirrus*, *E. caninus*, *E. trachycaulus*, and *E. mutabilis* (genomes SSHH) are morphologically vigorous but show a low frequency of bivalent formation, indicating distinctness between the Y and H genomes and thus precluding introgression between *E. dentatus* ssp. *ugamicus* and other species with the SSHH genomes (Dewey 1980a).

Sakamoto and Muramatsu (1966b) investigated pentaploid hybrids derived from crosses between the Japanese tetraploids *E. ciliaris*, *E. gmelinii*, and *E. yezoensis*, the Nepalese tetraploids *E. gmelinii* and *E. semicostatus* and the Japanese hexaploids *E. humidorus* and *E. tsukushiensis*. They found that the general characteristics of F1 hybrids were either intermediate between the parents or superior to those of the parents and that the hybrids were completely sterile. Chromosome pairing of the hybrids indicates that geographical isolation results in some chromosomal differentiation between the Japanese and Nepalese genomes. The other sterile pentaploid hybrids are produced from interspecific hybridization between tetraploid *E. lanceolatus*, *E. trachycaulus*, and *E. mutabilis* and hexaploid *E. alatavicus* and *E. batalinii* (Jensen et al. 1986). From studies of chromosome pairing of the hybrids, the genome constitutions of *E. alatavicus* and *E. batalinii* have been determined to be SSYYXX, respectively. Chromosome pairing in the pentaploid hybrids between *E. scabriglumis* and *E. trachycaulus* indicate that *E. scabriglumis* consists of the six genomes with the formula SSHHYY (Gupta et al. 1988).

Sakamoto and Muramatsu (1966a) and Sakamoto (1982) observed a high frequency of bivalent formation in the hexaploid hybrids derived from crosses between *E. humidum* and *E. tsukushiense* and between *E. dahuricus* and *E. tsukushiense*. They proposed that the genome constitution of the parental species possesses three different genomes and that the genomes of these species are closely related. The allohexaploid hybrids are morphologically vigorous but completely sterile. Hexaploid *E. alatavicus* (SSYYXX) hybridizes with hexaploid *E. drobovii* (SSHHYY) (Jensen et al. 1986). The high level of pairing in this hybrid is due to the presence of the Y genome rather than the H genome. The hybrids produced anthers with less than 2% stainable pollen and only 2 seeds in 150 spikes. Sterile interspecific hexaploid hybrids are produced from reciprocal crosses between the colchicine-induced octoploid *E. trachycaulus* and tetraploid *E. canadensis* (Aung and Walton 1989).

Complete sterility brings the interspecific hybrids to an immediate "dead end" unless fertility can be restored (Dewey 1967a).

Doubling of chromosome numbers of such hybrids offers the best prospect for restoring fertility. The induced amphiploids from *E. canadensis* x *E. caninus* are highly self-fertile (Dewey 1968d). Octoploid amphiploids (SSS'S'HHH'H') are derived by doubling the chromosome numbers of hybrids from crosses between *E. canadensis* and *E. trachycaulus* ssp. *subsecundus* (Dewey 1977b). Doubling of chromosome numbers initially restored fertility but drastically reduced vegetative vigor. Meiotic irregularities tended to increase in each succeeding generation. Sterility in the amphiploids is attributed to heterogenetic pairing between the closely related *E. canadensis* and *E. trachycaulus* ssp. *subsecundus*. Octoploid amphiploids derived from the completely sterile *E. trachycaulus* x *E. canadensis* hybrids show some restoration in fertility (Kumar and Walton, unpublished).

1.4.3. Intergeneric hybridization

The S and H genome of *Elymus* are found alone or together in *Peudoroegneria* (S), *Critesion* (H), *Elytrigia* (SX), and *Pascopyrum* (SHJN), thus providing a genomic basis for intergeneric hybridization with those four genera (Dewey 1984b; Gupta and Fedak 1985a,b; Wang and Hsiao 1986; Aung and Walton 1987b; Gupta et al. 1988). All of the amphiploids from hybrids between *Elymus* and *Pseudoroegneria* (Dewey 1974a; Asay and Dewey 1976; Asay et al. 1988) and F1 hybrids from crosses between *Pseudoroegneria* and *Elytrigia* (Dewey 1976; Asay and Hansen 1984, Asay and Dewey 1985) which share the S genome show good fertility and are meiotically very stable that the hybrids have been successfully used to develop new types of germplasm for forage improvement. *Elymus* can also hybridize with

Agropyron(P) (Napier and Walton 1982), *Psathyrostachys* (N) (Dewey 1967d), *Thinopyrum* (JE) (Napier and Walton 1983), *Leymus* (JN) (Bowden 1967; Dewey 1970; Sakamoto 1985), *Pascopyrum* (SHJN) (Dewey 1970), *Triticum* (ABD) (Mujeeb-kazi and Bernard 1982, 1985; Sharma and Gill 1983a,b; Mujeeb-kazi et al. 1984; Sharma and Baenziger 1986; Yen and Liu 1987), *Hordeum* (I) (Mujeeb-kazi and Rodriguez 1982; Fedak 1985; Mujeeb-kazi 1985), and *Secale* (R) (Hang and Francowiak 1984) that do not carry the S, H, or Y genomes.

These triploid, tetraploid, or pentaploid intergeneric hybrids often fail to show pairing between the parental genomes, with univalents at metaphase I, laggards during anaphase I, and micronuclei at the later stages of meiosis. As a result of the meiotic irregularities, most of meiocytes produced are unbalanced and therefore most pollen grains do not stain and there is little or no seed set where intergeneric hybrids do not share a common genome. Generally, the sterile intergeneric hybrids of *Elymus* are morphologically intermediate between the parents but are not promising for further breeding. Sterile intergeneric hybrids have been produced from crosses between *Elymus canadensis* as the female parent and *Leymus triticoides* and *L. secalinus* (Dewey 1970), *Critesion bogdanii* (Dewey 1971), *C. californicum* and *C. bulbosum* (Wang and Hsiao 1986), *Pseudoroegneria spicata* (Stebbins and Snyder 1956; Asay and Dewey 1976), *P. libanotica* (Dewey 1974b), *Triticum aestivum* (Mujeeb-kazi and Rodriguez 1982; Mujeeb-kazi and Bernard 1982, 1985; Yen and Liu 1987), *Hordeum vulgare* (Mujeeb-kazi and Rodriguez 1982), and *Secale cereale* (Hang and Franckowiak 1984) as the male parents. Among these hybrids, *P. libanotica* x *E. caninus* and

E. canadensis x *P. libanotica* amphiploids have potential as forage grasses, showing fertility and cytogenetic stability (Dewey 1974b; Asay and Dewey 1976).

Hexaploid hybrids from crosses between tetraploid *E. canadensis* (SSHH) and octoploid *L. cinereus* (JJJJNNNN) were mainly chlorophyll deficient and died (Dewey 1966a). A few survived and showed autosyndetic chromosome pairing [15.8 univalents (I) + 13.0 bivalents (II) + 0.04 trivalents (III)] of *L. cinereus* chromosomes, indicating that *E. canadensis* and octoploid *L. cinereus* had no chromosomes in common. This hexaploid hybrid strongly resembles *L. cinereus* and is completely sterile. No crossing barriers are encountered in this cross even though the parental species are not closely related, precluding the possibility of predicting species relationships on the basis of cross-compatibility or F1 hybrid fertility (Dewey 1966a).

1.5. Methodology of genome manipulation

1.5.1. Hybridization

The first step in the production of new allopolyploids involves the interspecific or intergeneric hybridization at the same or different ploidy levels of the parental species. A series of autopolyploids can be produced by interploidy hybridization following doubling of chromosome numbers. There are barriers which prevent the successful hybridization between two incompatible species or between different ploidy levels of the same species. Several techniques have been devised to overcome the barriers to successful production of viable hybrids (Brar and Khush 1986). These techniques include use of exogenous growth substances (GA or IAA) and immunosuppressants (EACA), embryo rescue, and bridging species. The traditional application of embryo culture to crop improvement has been documented for many crop species by Williams et al. (1987). Use of hormones (generally 50-75 ppm gibberellic acid) and *in vitro* culture of 10-15 days-old hybrid embryos on a nutrient medium (MS, B5, or LS) has been effectively employed to recover immature and undifferentiated embryos in wide crosses among Triticeae species (Napier and Walton 1983; Sharma and Gill 1983a; Mujeeb-kazi and Bernard 1985; Gupta et al. 1988). Callus formation of undifferentiated hybrid embryos and subsequent plant regeneration is an alternative to conventional embryo rescue (Larkin 1985; Merkle et al. 1988).

1.5.2. Colchicine treatment

Colchicine is not only a mitotic poison that inhibits spindle formation but is an ideal tool for the study of growth, and is the best polyploidizing agent for use in plants (Eigsti and Dustin 1955). Different colchicine techniques have been described for the production of amphiploids from interspecific and intergeneric hybrids involving *Triticum*, *Aegilops*, *Secale*, and *Agropyron* (Bell 1950; Kaltsikes 1974). Colchicine solutions of 0.1 to 0.4% applied for 12 to 48 hours appear to be suitable for inducing polyploidy in *Agropyron cristatum* (Tai and Dewey 1966). Ahloowalia's investigation (1967) on induction of tetraploid *Lolium perenne* reveals different responses to colchicine treatment of different varieties, and mutagenic effects in addition to doubling chromosome numbers. Satisfactory techniques have been developed whereby vegetative grass tillers can be successfully treated with 0.2% colchicine plus 2% dimethy-sulphoxide (DMSO) to produce polyploid sectors (Morgan 1976). Adding a wetting agent (Tween 20) and GA3 to colchicine, plus DMSO solution, has been used to double chromosome numbers of haploid barley (Thiebaut and Kasha 1978). Use of earlier development stages (three leaf stage), higher temperature (32 °C), and addition of N-6-benzyl adenine (BA) has been used to increase the extent of doubling within plants (Thiebaut et al. 1979). In one method, hybrid callus which was subcultured three times on solid subculture medium was transferred to the same medium supplemented with filter sterilized colchicine at 20mg/L and incubated at 10 °C for ten days in darkness prior to induction of plant regeneration (Nakamura et al. 1981).

1.5.3. Somatic tissue culture

Several grasses and cereals regenerated from the cultured callus of immature embryos, immature inflorescences, or young leaves showed variation in chromosome number or structure(Vasil 1987). A few polyploid lines have been obtained from tissue culture of durum wheat (Bennici and D'Amato 1978), barley (Gaponenko et al. 1988), rice (Sun et al 1983), maize (Lee and Phillips 1987), barnyardgrass (Takahashi et al. 1984), pearl millet (Swedlund and Vasil 1985), tall fescue (Einzenga 1989), Italian ryegrass x tall fescue hybrids (Kasperbauer et al. 1979), and *Triticum* x *Secale* or *Hordeum* hybrids (Fedak 1984; Fedak and Grainger 1986).

Chromosomal rearrangements have also been observed in some regenerants from somatic cells and tissues of interspecific hybrids of barley (Orton 1980; Jorgensen and Andersen 1989) and *Lolium* (Ahloowalia 1983), and intergeneric hybrids including *Triticum, Secale, Aegilops,* and *Hordeum* (Armstrong et al. 1983; Fedak and Sampson 1983; Chu et al. 1984; Lapitan et al. 1984; Fedak and Grainger 1986; Gupta and Fedak 1988; Jahier and Tanguy 1988).

Chromosome instability during *in vitro* culture of plant tissue occurs both in cultured callus cells and regenerated plants. Several mechanisms that may produce chromosome variability were found to exist in studies of the nuclear cytology of cultured tissues (D'Amato 1985). Chromosome aberrations have been presumed to occur randomly by chromosomal breaking and rejoining, and by DNA amplification in heterochromatic regions (McCoy et al. 1982; Larkin 1987; Lee and Phillips 1987). Media components, explant, culture age, and genotype all affect the occurrence of cytological aberrations (Vasil 1987; Larkin 1987). Chromosomal variation in culture is known to include changes in chromosome number (polyploidy and aneuploidy) and structure (deletion, duplication, inversion and translocation). Pre-existing chromosome variation, nuclear fragmentation, endoreduplication or endomitosis, and abnormality of mitosis are assumed to influence the variation in chromosome number during tissue culture (Evans et al. 1984). When detailed analysis has been possible, structural alterations are detected more frequently than changes in chromosome number (Lee and Phillips 1987). Small changes in chromosome structure could alter expression and genetic transmission of specific genes perhaps by deletion or duplication of one copy of a gene (Evans 1989).

Generally, tissue culture has been considered as a tool for genome manipulation (Fedak 1984; Fedak and Grainger 1986), enhancement of alien gene introgression in wide crosses (Orton 1980; Lapitan et al. 1984; Brar and Khush 1986; Larkin 1987), generation of aneuploid stocks for mutant selection (McCoy et al. 1982) and for cytogenetic studies (McCoy et al. 1982; Chen et al. 1987).

1.6. Objectives

The objective of this study was to combine genomes of *Elymus canadensis* with those of *Psathyrostachys juncea* and *Secale cereale* and to manipulate the genome constitution of *Elymus canadensis* in relation to forage improvement. An outline of the crossing program for the study is presented in Fig. 1.1 (chapter 2), Fig 1.2 (chapter 3), and Fig. 1.3 (chapters 4, 5, and 6).

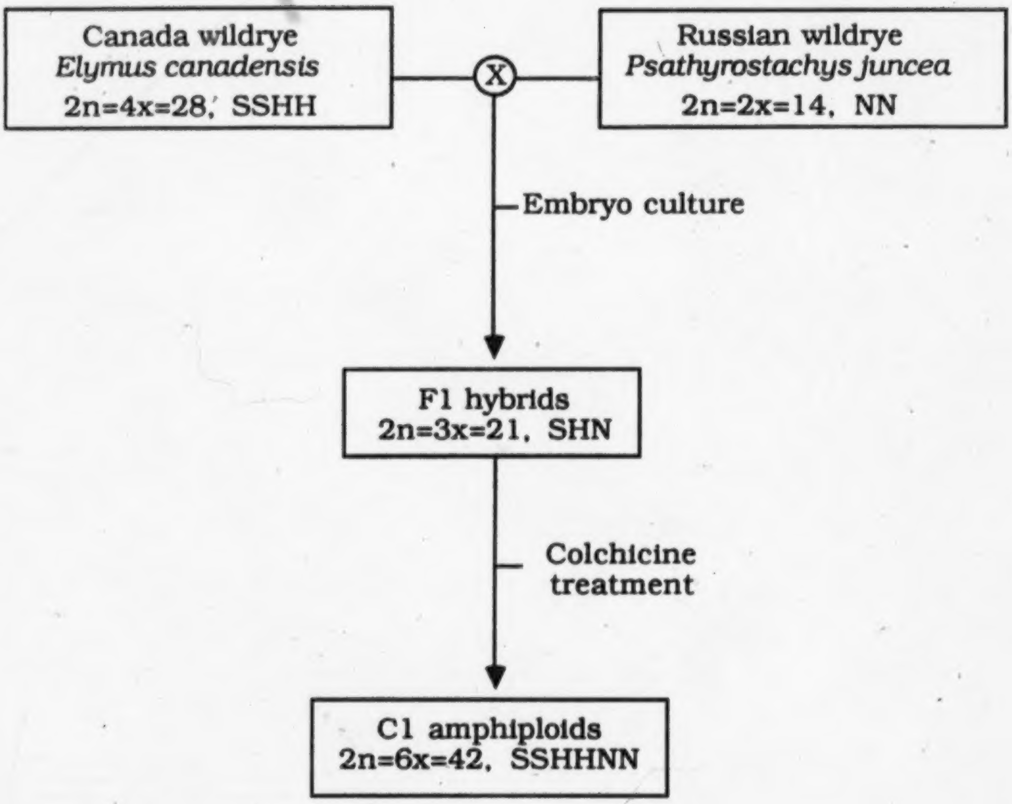

Fig. 1.1. Diagram of hybridization between Canada wildrye and Russian wildrye

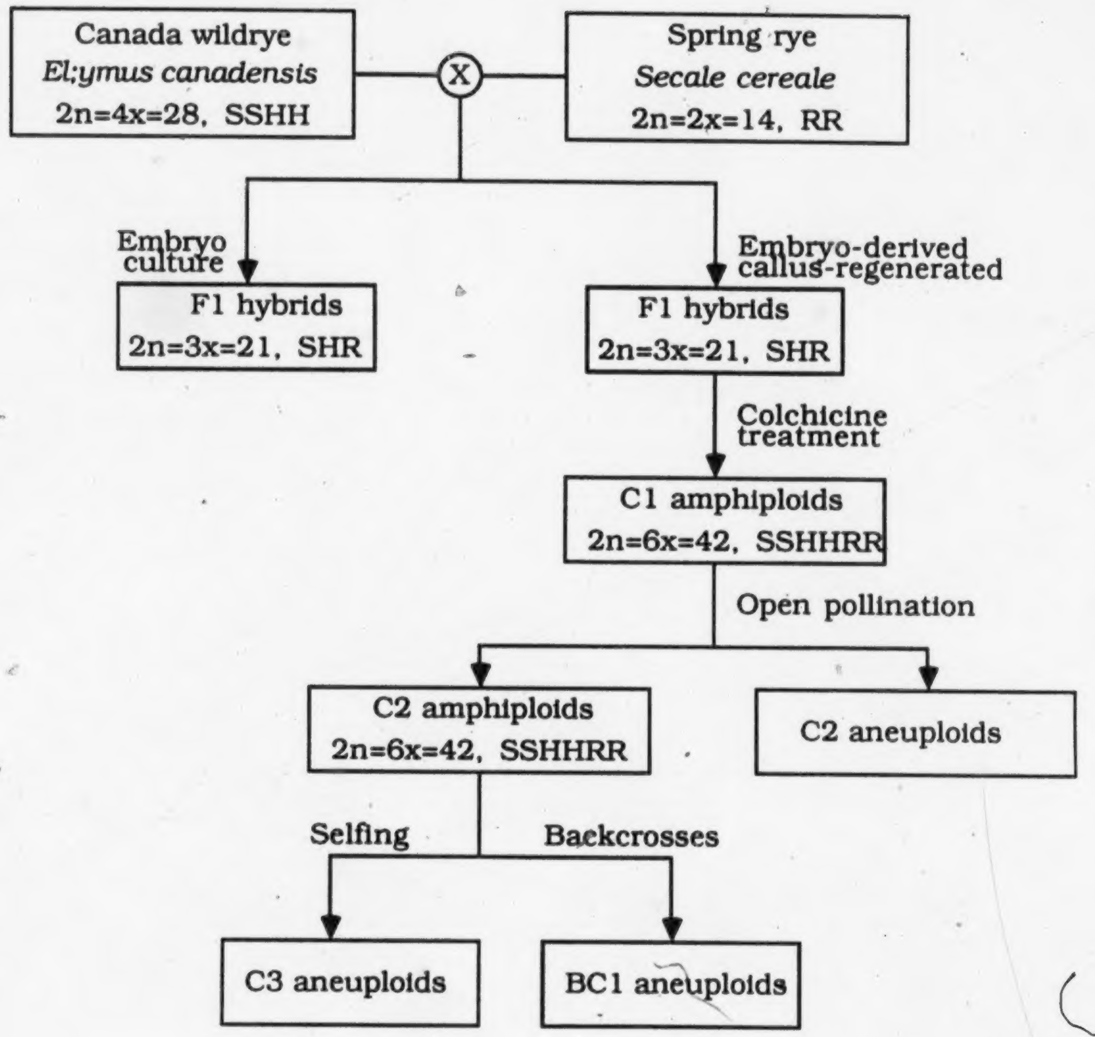

Fig. 1.2. Diagram of hybridization between Canada wildrye and Spring rye

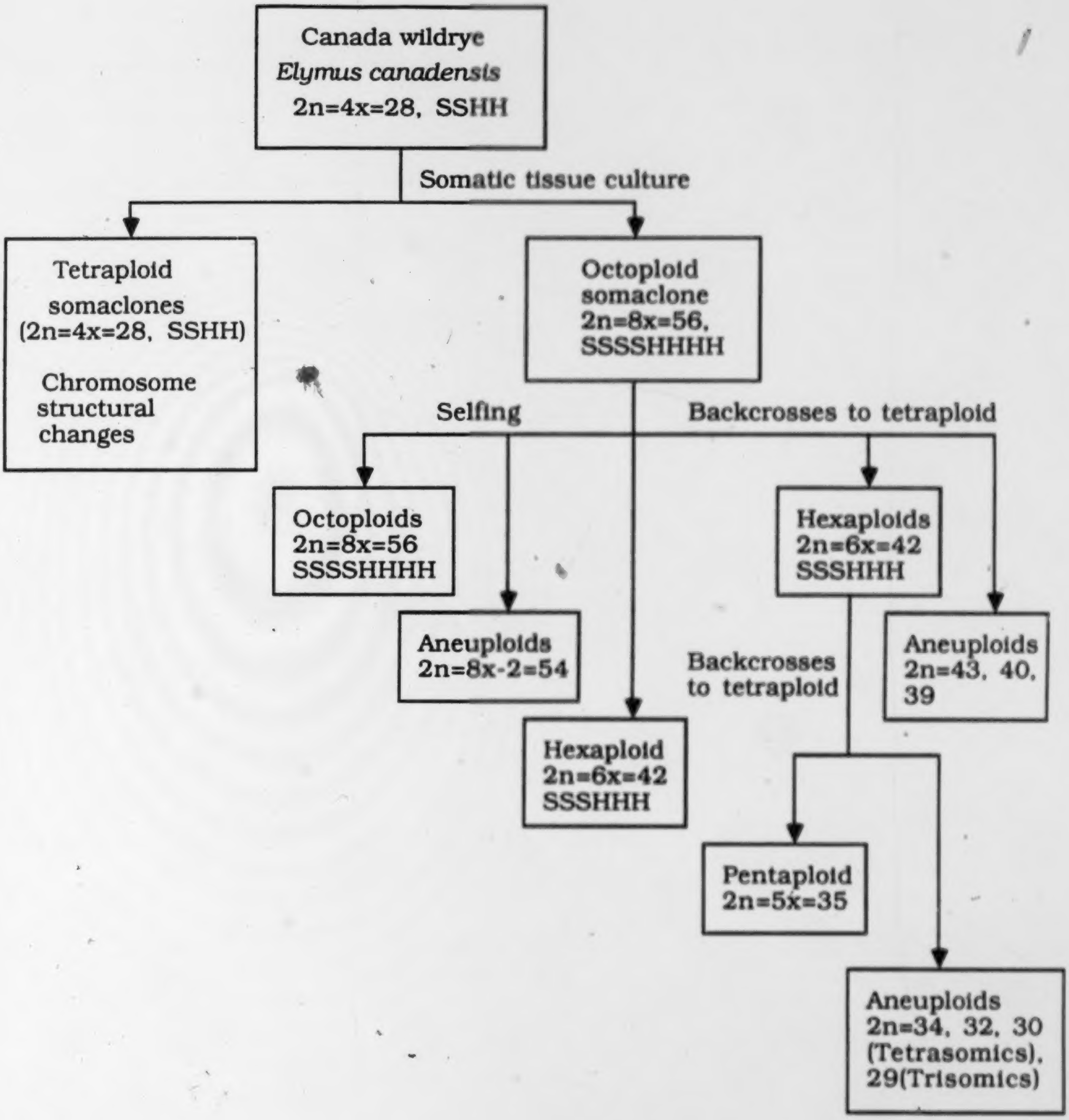
Canada wildrye
Elymus canadensis
2n=4x=28, SSHH
Somatic tissue culture
Tetraploid somaclones (2n=4x=28, SSHH)
Chromosome structural changes
Octoploid somaclone 2n=8x=56, SSSSHHHH
Selfing
Backcrosses to tetraploid
Octoploids 2n=8x=56 SSSSHHHH
Aneuploids 2n=8x-2=54
Hexaploid 2n=6x=42 SSSHHH
Hexaploids 2n=6x=42 SSSHHH
Aneuploids 2n=43, 40, 39
Backcrosses to tetraploid
Pentaploid 2n=5x=35
Aneuploids 2n=34, 32, 30 (Tetrasomics), 29(Trisomics)

Fig. 1.3. Diagram of genome manipulation of Canada wildrye using tissue culture and interploidy hybridization

1.7. References

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2. INTERGENERIC HYBRIDS FROM CROSSES BETWEEN *Elymus canadensis* AND *Psathyrostachys juncea* AND AMPHIPLOIDS DERIVED FROM THESE HYBRIDS[1]

2.1. Introduction

Canada wildrye (*Elymus canadensis* L., 2n=4x=28, SSHH) is a North American native forage grass which is sometimes cultivated for its high forage yield under cool and moist conditions. Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski, 2n=2x=14, NN] is an Asian native species which was introduced into North America and subsequently found to be an excellent pasture grass for the dry southern parts of Canadian prairies and for the semiarid rangelands of the Western mountains and the Northern Great Plains of the United States (Walton, 1983; Asay & Knowles, 1985).

The two species are different from each other in forage yield, quality, and their tolerance to environmental stresses such as drought, coldness, and soil salinity. Canada wildrye is more productive and establishes more quickly, but has lower quality and is less tolerant of environmental stresses than Russian wildrye. Hence, the synthesis of these species, or gene transfer from one species into another by hybridization, may produce desirable new genotypes for forage improvement.

With this prospect in view, an attempt was made to produce intergeneric hybrids by crossing *E. canadensis* with *P. juncea* and produce amphiploids from them. This study describes morphological and cytological characteristics of these hybrids and polyploids.

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[1] A version of this chapter has been published. Park & Walton 1989. Euphytica (in press).

2.2. Materials and methods

2.2.1. Plant materials

Three accessions of Canada wildrye, 'Beaverlodge' (Beaverlodge 211), 'Canada' (PI-372539), and 'Montana' (PI-232249), were obtained from Dr. Taing Aung in the Department of Plant Science, University of Alberta. These accessions were used in 1986 as the female parents in crosses with Russian wildrye cv. 'Mayak' as the male parent.

2.2.2. Hybridization

The plants were grown in 12.5 cm pots in the greenhouse. The florets of Canada wildrye were emasculated 2 days before anthesis and hand-pollinated with Russian wildrye pollen. In order to stimulate embryo development, 0.2% GA3 was sprayed on the florets of Canada wildrye two days before and after anthesis.

2.2.3. Embryo rescue

Immature embryos (18 days) were excised, sterilized in a 4%-6% sodium hypochlorite solution for 10 minutes, and cultured on MS medium (Murashige and Skoog 1962). At the three-leaf stage, seedlings were transplanted from test tubes to soil in the greenhouse.

2.2.4. Amphiploid production

Some of the hybrid plants were split into individual tillers and their roots were removed. Each tiller was immersed in 0.2% aqueous colchicine for 24 hours and rinsed in tap water for four hours. Amphiploid sections were isolated from the mixoploid clone at anther dehiscence.

2.2.5. Morphological investigation

At maturity, the morphological characteristics of the parents, F1 hybrids, and amphiploids were studied. Plant height, leaf size, leaf area, tiller number, dry weight, and leaf/stem ratio were recorded. Pollen fertility was determined from pollen grains stained with 1.5% acetocarmine solution. Seed fertility was determined as the proportion of florets per spike that contained seed.

2.2.6. Cytological observation

For cytological observations, root-tips were kept in cold water at 2 °C for 24 hours and then fixed in three parts ethanol to one part acetic acid. Young spikes were fixed in Carnoy's solution (6:3:1 ethanol-chloroform-acetic acid). Squash preparations of mitosis and meiosis were made in 1.5% acetocarmine solution.

2.3. Results

2.3.1. Production of F1 hybrids and amphiploid

Three hundred and twenty embryos were obtained from the 1027 florets pollinated (31.2%). Cross compatibility, germination rate, and number of plants that survived in each cross are shown in Table 2.1. Two accessions of Canada wildrye had a high cross compatibility of 47.8% ('Montana') and 51.9% ('Beaverlodge'), whereas 'Canada' exhibited low compatibility (14.4%). The embryos obtained from the cross of 'Montana' with Russian wildrye showed the highest rate of germination (48.3%) and survival (71.0%). One amphiploid plant was obtained from the 17 tillers of two plants of Canada wildrye 'Montana' x Russian wildrye hybrids (designated as MR) which were treated with colchicine.

2.3.2. Morphological characteristics

Table 2.2 shows the morphological characteristics and pollen stainability of the parents, F_1 hybrids, and amphiploid. Canada wildrye 'Beaverlodge' x Russian wildrye hybrids (designated as BR) were not vigorous and necrotized before heading. However, 'Canada' x Russian wildrye hybrids (designated as CR) and 'Montana' x Russian wildrye hybrids (MR) exhibited hybrid vigor but generally resembled Russian wildrye in morphology. Unlike the parent Russian wildrye, the hybrids have both basal leaves and stem leaves. Canada wildrye 'Canada' x Russian wildrye hybrids (CR) and MR showed superiority in plant height, leaf area, and dry weight over the parents (Fig. 2.1a-d). Dry weights of CR and MR were respectively 8.6% and 6.4% higher than that of Canada wildrye. Leaf size, length of spike (Fig. 2.1f) and spikelet (Fig. 2.2a-c), tiller number, and leaf/stem ratio of the hybrids were intermediate between the parents. Pollen stainability of all hybrids was extremely low, ranging from 0.3% to 3.5% (Fig. 2.2d). All hybrids were completely sterile. The amphiploid plant was morphologically similar to the F1 hybrid (Fig. 2.1e) but showed 48.9% pollen stainability (Fig. 2.2e) and produced 26 seeds (16 shriveled and 10 plump) from 225 florets of three spikes examined (11.6%).

2.3.3. Cytological behavior

With the exception of one MR plant (designated as MR1) having 22 chromosomes, all hybrids were triploids with 21 chromosomes (Fig. 2.3a). One amphiploid plant was an allohexaploid ($2n=6x=42$) (Fig. 2.3b). Chromosome pairing in the pollen mother cells (PMCs) of the F1 hybrids and amphiploid at the first metaphase is set out in Table 2.3. Mean number of chromosome association in the hybrids at metaphase I was 16.91 univalents (I) + 2.03 bivalents (II) + 0.06 trivalents (III) + 0.02 quadrivalents (IV) (Fig. 2.3c & d). MR ($2n=3x=21$) showed a higher frequency of bivalents than BR, CR, and MR1 ($2n=3x+1=22$). In the hybrids, the range of bivalents per cell was from 0 to 8 and 77% of them were loosely-paired homologous. The amphiploid PMCs showed an increase in chromosome pairing at diakinesis and metaphase I (Fig. 2.3e & f). Two percent of the meiocytes examined contained 21 bivalents at metaphase I (Fig. 2.3e). The mean frequencies of I, II, and III were 5.85, 18.00, and 0.07, respectively. Ring-type bivalents increased 54% in the amphiploid. Chiasma frequency of the hybrids averaged 2.6 per cell but that of the amphiploid was 27.7 per cell. Unequal chromosome disjunctions (Fig. 2.3g), lagging chromosomes (Fig. 2.3h), and chromosome bridges (Fig. 2.3h) were common at anaphase I and II in the F1 hybrids and amphiploid. Micronuclei (Fig. 2.3i) of variable size and number, and abnormal cytokinesis, were occasionally observed at telophase and at the tetrad stage of the hybrids and amphiploid.

2.4. Discussion

Canada wildrye hybridizes readily with several other *Elymus* and some *Leymus* species (Dewey 1967a). Russian wildrye also crosses with several tetraploid *Elymus* species giving a low frequency of seed set and hybrid development (Dewey 1970, 1972a & b). The cross between Canada wildrye and Russian wildrye has not been reported previously. Compared to other *Elymus* and *Leymus* species that Dewey used to cross with Russian wildrye, Canada wildrye used in this cross showed a high cross compatibility. This is assumed to be due to the action of gibberellic acid (GA3) on embryo development.

The increase in dry weight of CR and MR plants, in spite of the intermediate phenotypes between the parents for some morphological characters, could be attributed to the increased leaf area of the hybrids. Although F_1 hybrids produced only defective pollen and no seed, the anthers of the amphiploid plant burst well and showed a significantly high rate of pollen stainability. The fertile pollen restored significant seed fertility to the amphiploid. Pollen stainability and seed set (seeds/spike) of hexaploid amphiploids (2n=6x=42, SSSSHH) between *Elymus canadensis* and *Pseudoroegneria libanotica* averaged 63.3% and 40.5% respectively from the C_1 through C_3 generation while those of the hexaploid amphiploid between *E. canadensis* and *P. spicata* averaged 19.6% and 6.6%, respectively (Asay and Dewey 1976). On the other hand, the hybrids from crosses between *E. canadensis* with some other *Elymus* or *Leymus* species were completely sterile in spite of their genomic affinity (Dewey 1970, 1972a & b). Such sterility was assumed to be due to structural chromosome differences between the closely related genomes. The sterility and lack of plant vigor made the intergeneric hybrids unsuitable for forage improvement. However, amphiploids derived from *E. canadensis* x *P. libanotica* hybrids are promising for further breeding in terms of fertility and cytological stability (Asay and Dewey 1976).

Canada wildrye is a self-fertilizing allotetraploid with a genome constitution of SSHH. Mainly ring-type bivalents of the fourteen are found at metaphase I of Canada wildrye. Russian wildrye is an outcrossing diploid (genome NN) which forms seven bivalents at metaphase I. The allotriploid hybrids with the S, H, and N genomes showed very low chromosome pairing, averaging 2 bivalents per cell. The low frequency and loose- rod type of bivalents indicate that neither the S nor H genomes of Canada wildrye pair with the N genome of Russian wildrye. The hybrids from crosses between *Elymus scribneri* (SSHH) and *Psathyrostachys juncea* (NN) exhibited very little chromosome pairing, also indicating the distinctness of the S, H, and N genomes (Dewey 1967b). The hybrids from crosses between diploid *P juncea* with the other seven tetraploid *Leymus* species (*L. cinereus*, *L. triticoides*, *L. karataviensis*, *L. secalinus*, *L. multicaulis*, *L. racemosus*, and *L. innovatus*) showed a relatively high frequency of bivalents ranging from 3.94 to 6.81 per cell (Dewey 1970, 1972a & b). These differences in chromosome pairing of the SHN triploid hybrids may be attributed to the different extent and randomness of intragenomic or intergenomic pairing. The SH hybrid from the cross between

Pseudoroegneria spicata (SS) and *Critesion violaceum* (HH) and the dihaploid (SH) of *Elymus canadensis*, of which H genome donor is unknown, indicate the intragenomic or intergenomic pairing by a very low frequency of bivalents (Wang & Hsiao 1986; Asay et al. 1987). Chromosome configurations of the hybrid (SN^h) from crosses between *Pseudoroegneria spicata* ssp. *inermis* (SS) and *Psathyrostachys huashanica* (N^hN^h) and the hybrid (HN) between *Critesion violaceum* (HH) and *Psathyrostachys juncea* (NN) show that little homology exists between the S and N^h genomes or between the H and N genomes (Wang 1986, 1987). In this study, the appearance of several bivalents or multivalents in a few triploid PMCs indicates some possibility of intergenomic pairing between partially homologous chromosomes or a random intragenomic pairing. Although the amphiploid consisted of six genomes, SSHHNN, bivalents predominated reflecting intragenomic pairing between homologous chromosomes. This supports the hypothesis that the genomes S, H, and N are distinct. However, the occasional trivalent formation indicates a small capability of intergenomic pairing in the amphiploid.

In summary, hybridization of *E. canadensis* with *P. juncea* offers limited prospects of gene transfer due primarily to lack of homology between the parental genomes. However, a low frequency of homoeologous pairing does offer the possibility of gene transfer by producing alien chromosome addition lines and combining with use of irradiation and tissue culture techniques. Since this amphiploid does not exist in nature, it is a promising source of germplasm for forage improvement provided it is genetically and cytologically stable in future generations.

Table 2.1 Number and percent (in parentheses) of hybrid embryos, seedlings, and surviving plants from a cross of *Elymus canadensis* x *Psathyrostachys juncea*

Cross combinations	Nº of florets pollinated	Nº of embryos cultured	Nº of embryos germinated	Nº of seedlings transplanted	Nº of plants survived
Elymus canadensis 'Beaverlodge' x *Psathyrostachys juncea*	77	40 (51.9)	12 (30.0)	10 (83.3)	6 (60.0)
Elymus canadensis 'Canada' x *Psathyrostachys juncea*	521	75 (14.4)	13 (17.3)	11 (84.6)	4 (36.4)
Elymus canadensis 'Montana' x *Psathyrostachys juncea*	429	205 (47.8)	99 (48.3)	62 (62.6)	44 (71.0)
Total	1027	320 (31.2)	124 (38.8)	83 (66.9)	54 (65.1)

Table 2.2. Morphological characteristics of parents, F1 hybrids, and amphiploid from a cross of *E. canadensis x P. juncea*

Genotype	Nº of plants	Plant height (cm)	Leaf length (cm)	Leaf width (mm)	Spike length (cm)	Nº of tillers	Leaf area (cm2)	Dry weight (g)	Leaf/ stem ratio
E. canadensis									
Beaverlodge	5	97.3	23.5	90	19.0	28.4	7.72	13.0	0.93:1
		(3.04)	(0.45)	(0.71)	(0.60)	(0.62)	(0.37)	(0.80)	
Canada	5	105.0	26.8	92	18.7	25.1	8.05	12.8	0.98:1
		(2.60)	(1.08)	(0.41)	(0.25)	(0.71)	(0.30)	(0.91)	
Montana	5	103.2	28.8	93	19.3	27.0	8.16	14.0	0.95:1
		(6.40)	(1.43)	(0.41)	(0.56)	(1.00)	(0.33)	(0.50)	
P. juncea									
Mayak	1	101.5	48.4	50	12.9	36.0	7.36	13.5	1.99:1
F1 hybrids									
Beaverlodge x	2	32.7	18.8	60	5.8	28.6	2.15	3.40	2.70:1
Mayak(BR)		(3.90)	(0.30)	(1.00)	(0.20)	(1.40)	(0.21)	(0.60)	
Canada x	4	113.5	34.0	70	15.9	28.0	9.74	15.2	1.41:1
Mayak(CR)		(2.53)	(1.96)	(1.08)	(0.28)	(1.10)	(1.27)	(0.37)	
Montana x	9	120.0	33.8	70	17.1	25.0	8.86	14.9	1.28:1
Mayak(MR)		(5.96)	(0.73)	(0.90)	(0.59)	(1.90)	(0.41)	(0.62)	
MR Amphiploid	1	110.2	32.5	70	15.7	26.0	8.20	13.9	1.25:1

Standard errors in parentheses

Table 2.3. Chromosome associations of hybrids and amphiploid derived from the cross between *E. canadensis* and *P. juncea* at metaphase I

Genotype	Nº of plants	Nº of somatic chromosomes	Nº of cells	Mean chromosome associations (range)				Ring bivalents (%)	Frequency of chiasma
				I	II	III	IV		
BR	1	21	56	16.94 (7-21)	1.97 (0-5)	0.04 (0-1)		20.1	2.12
CR	2	21	152	16.93 (8-21)	1.86 (0-5)	0.12 (0-1)	0.04 (0-2)	11.3	1.91
MR	5	21	569	15.72 (2-21)	2.41 (0-8)	0.07 (0-2)	0.01 (0-1)	32.8	3.31
MR1	1	22	151	18.06 (2-22)	1.89 (0-8)	0.01 (0-1)	0.01 (0-1)	27.2	3.26
Amphiploid(MR)	1	42	97	5.85 (1-14)	18.00 (14-21)	0.07 (0-1)		54.0	27.72

Fig. 2.1. Plant and spike morphology of *E. canadensis* x *P. juncea* hybrids. **a.** *E. canadensis* accession 'Montana'. **b.** *P. juncea* cv. 'Mayak'. **c.** 'Montana' x 'Mayak' hybrid (MR). **d.** 'Canada' x 'Mayak' hybrid (CR). **e.** MR amphiploid. **f.** spikes of *E. canadensis*, F_1 hybrid (MR), and *P. juncea* (from left).

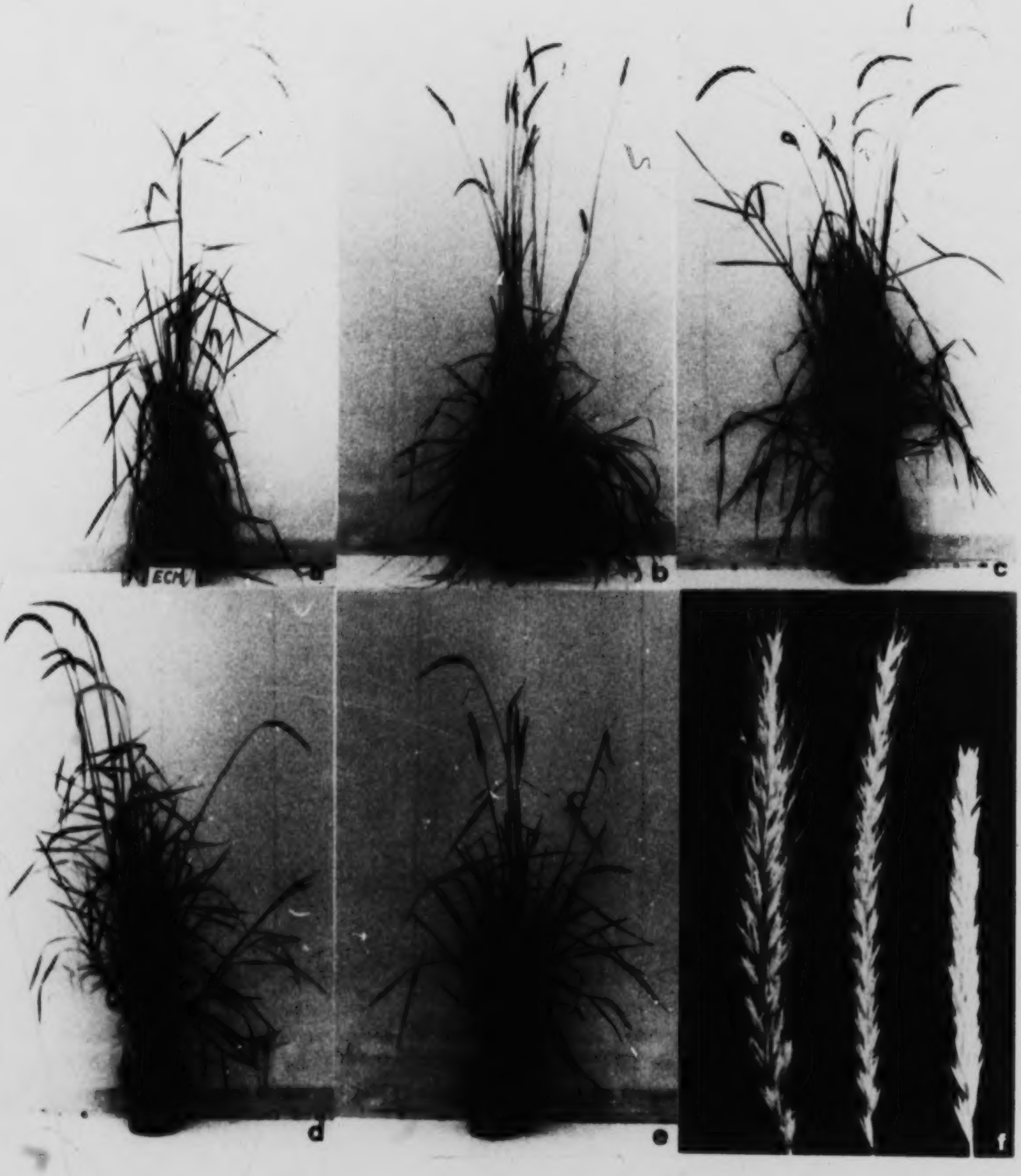
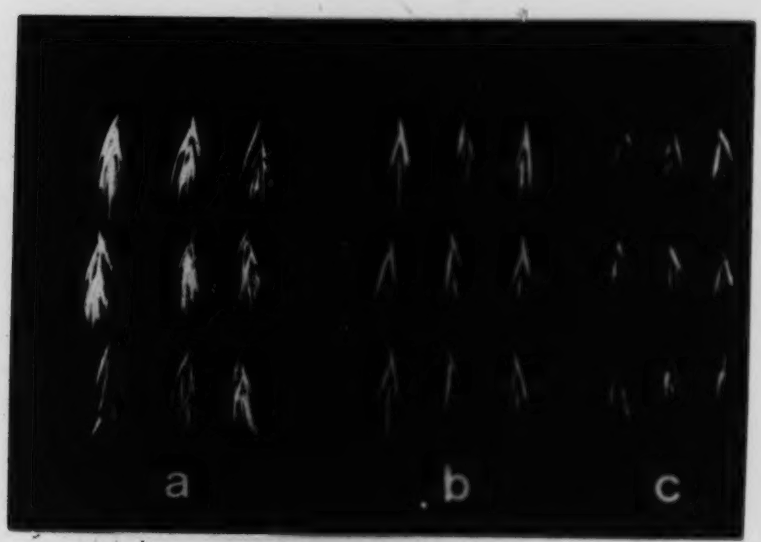

ECM
a
b
c
d
e
f

Fig. 2.2. Spikelets and pollen of *E. canadensis* x *P. juncea* hybrids. **a.** *E. canadensis*, **b.** F_1 hybrid (MR), **c.** *P. juncea*, **d.** nonstainable pollen of F_1 hybrid (MR), **e.** stainable pollen of amphiploid.


a
b
c

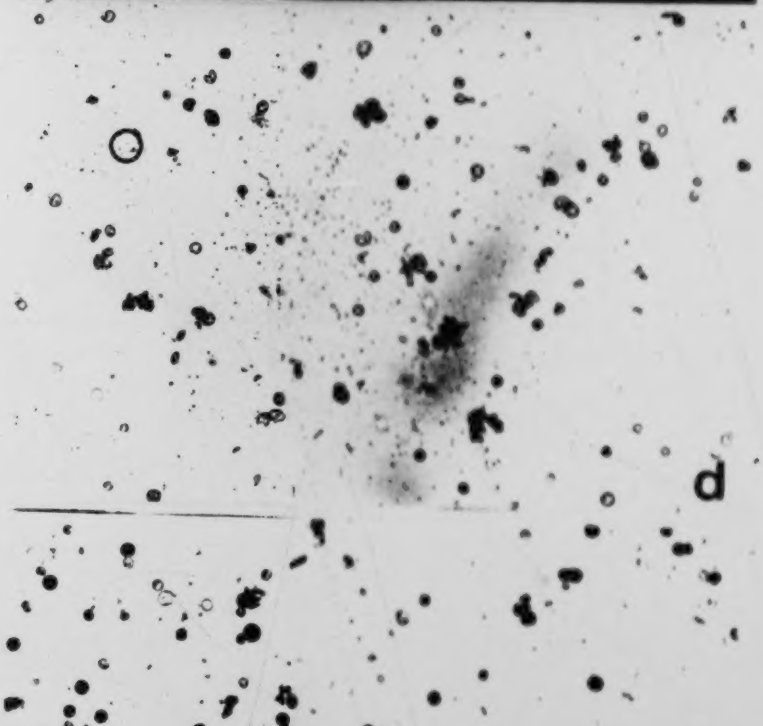
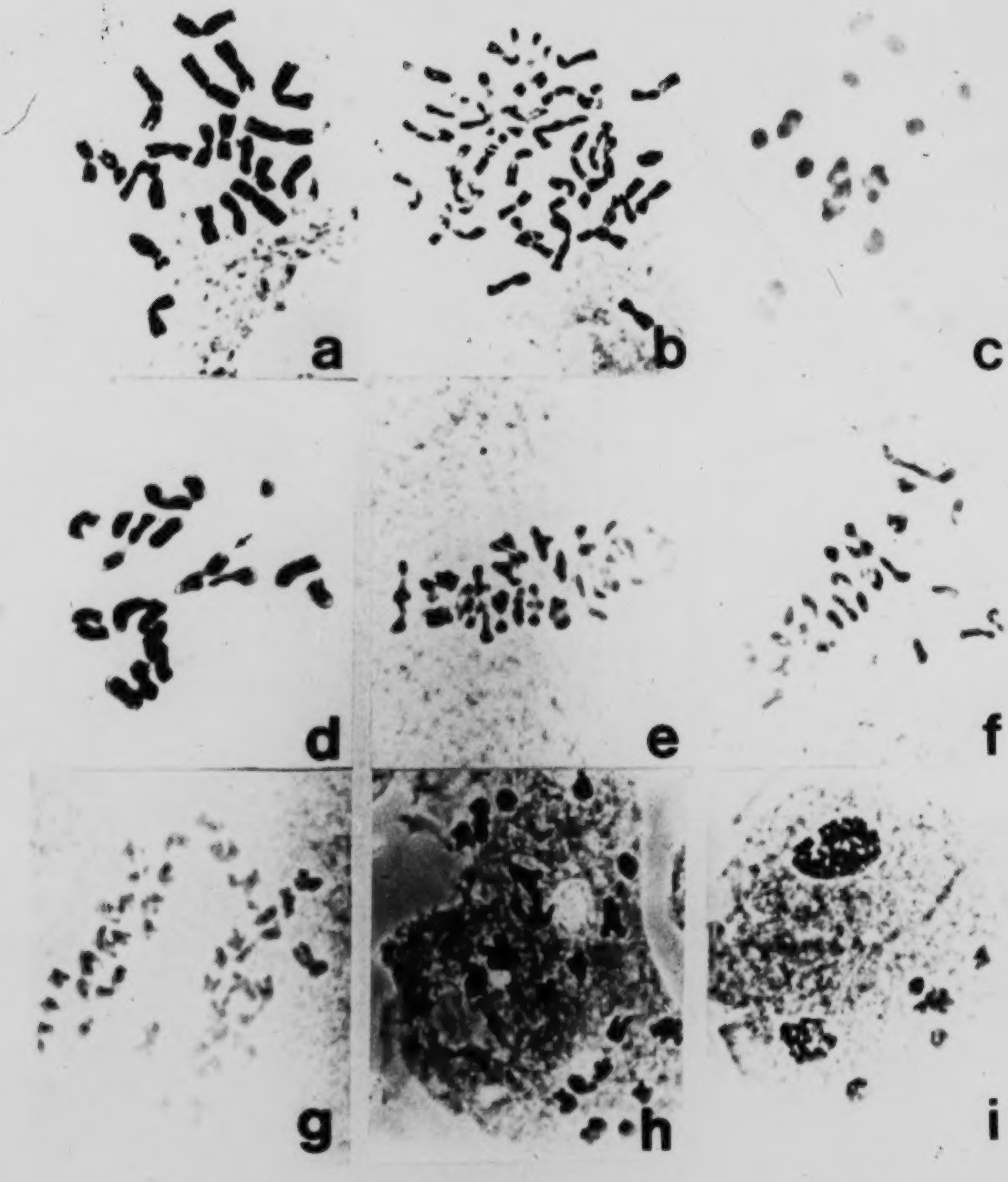

d

Fig. 2.3. Somatic chromosome complements and meiotic chromosome behavior of *E. canadensis* x *P. juncea* hybrids. **a.** somatic chromosomes of F_1 hybrid (MR) (2n=3x=21). **b.** somatic chromosomes of amphiploid (2n=6x=42). **c.** 15 I + 3 II in a F_1 PMC at MI. **d.** 16 I + 1 II + 1 III (arrow) in a F_1 PMC at MI. **e.** 21 II in an amphiploid PMC at MI. **f.** 8 I + 17 II in an amphiploid PMC at MI. **g.** unequal chromosome segregation in an amphiploid PMC at AI. **h.** lagging chromosomes and chromatid bridge in a F_1 hybrid at AI. **i.** abnormal cytokinesis and micronuclei in a F_1 hybrid at the tetrad stage.


a
b
c
d
e
f
g
h
i

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3. EMBRYO DERIVED-CALLUS-REGENERATED HYBRIDS AND THEIR COLCHICINE-INDUCED AMPHIPLOIDS FROM CROSSES BETWEEN *Elymus canadensis* AND *Secale cereale* [1]

3.1. Introduction

Canada wildrye (*Elymus canadensis* L.) is a North American native perennial grass with agronomic characteristics of high forage yield and moderate quality (Walton 1983). Spring rye (*Secale cereale* L.) is an annual species used as a reliable cereal crop, forage crop, or a cover crop over a wide geographic range (Stoskopf 1985).
From the standpoint of forage production, the poor tillering and foliage of spring rye makes it unsuitable for hay or pasture production. Canada wildrye has coarse leaves and poor forage quality when it matures but it is winterhardy and drought resistant (Walton 1983). Accordingly, hybrids and amphiploids from crosses between these two species may combine the advantage of both.

Allopolyploids have played a vital role in genome reconstruction and evolution of many crop species. Hybridization between related species of Triticeae followed by doubling of the chromosome number of the hybrids has produced new grain and forage species (Dewey 1979, 1984; Lukaszewski and Gustafson 1987; Asay et al. 1988). One of the main objectives in development of the amphiploids has been to search for strains with improved meiotic stability and increased fertility (Shigenaga et al. 1971). However, poor fertility, limited agronomic value, and cytological instability among the amphiploid progeny have limited their use in breeding new species (Asay and Dewey 1976, Muntzing 1979).

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[1] A version of this chapter has been partly published, Park & Walton 1989. Theor. Appl. Genet. (in press), and partly accepted for publication, Park & Walton 1989. Plant Breeding (in press).

Previously, Canada wildrye was hybridized with wheat (Mujeeb-Kazi and Bernard 1985; Yen and Liu 1987), barley (Mujeeb-kazi and Rodriguez 1982), and rye (Hang and Franckowiak 1984) to breed disease resistant cereal species. Intergeneric hybrids were produced by embryo rescue. Callus formation and plant regeneration from immature hybrid embryos has been regarded as an alternative way to develop hybrid plants (Larkin 1985; Merkle et al., 1988). In this study, the undifferentiated hybrid embryos in conventional embryo culture were subjected to callus formation and plant regeneration. This study describes the morphological and cytological characterization of the F1 hybrids and amphiploids from crosses between Canada wildrye and spring rye.

3.2. Materials and methods

3.2.1. Plant materials

Canada wildrye (*Elymus canadensis* L., 2n=4x=28, SSHH) accession, 'Montana (PI-232249)', was provided by Dr. Taing Aung, University of Alberta, Edmonton. Spring rye (*Secale cereale* L., 2n=2x=14, RR) cv 'Gazelle' was provided by Hanns Jahn, University of Alerta Edmonton Research Station.

3.2.2. Hybridization

Canada wildrye was used as the female parent and spring rye as the male parent. Canada wildrye was emasculated and then hand-pollinated with pollen of spring rye. At 24 h and 48 h before and after pollination, the pollinated florets were sprayed with GA3 (50 ppm).

3.2.3. Embryo culture

Fifteen days after pollination, the immature embryos were excised, sterilized in 4%-6% sodium hypochlorite solution for 5 min, rinsed 3 times with sterilized water, and then cultured on MS medium in 15 mm x 180 mm test tubes. The seedlings differentiated from the cultured embryos were transplanted at the three-leaf stage into soil in the greenhouse.

3.2.4. Embryo derived-callus culture and plant regeneration

Undifferentiated embryos were transferred from the conventional embryo culture (3.2.3) to the MS callus culture medium containing 2 mg 2,4-D per litre. Calli were initiated in the dark for the first month and then subcultured monthly on fresh MS callus culture medium in a growth chamber at 24+1 ^{o}C and 110 uE sec^{-1}cm^{2} fluorescent light. Three month-old calli were transferred to hormone-free MS medium for plant regeneration. The regenerated plantlets under the same culture conditions as for callus culture were moved to soil in the greenhouse at the three-leaf stage.

3.2.5. Amphiploid production

The regenerated hybrid plants were split into individual tillers to double their somatic chromosome complements using colchicine. Four hundred and eighty tillers were trimmed and immersed in the mixture of 0.2% colchicine and 4% DMSO for 24 h. After a 6-hour rinse in tap water, the tillers were potted in the greenhouse and the surviving plants were examined for somatic chromosome numbers, dehiscent anthers, dusty pollen, and seeds in florets, Doubled sectors (C_1) were separated from the mixoploid clones. Open-pollinated seeds (C_2) were collected from the C_1 clones and the C_2 seedlings were grown in the greenhouse. Four spikes of one C_2 ($2n=6x=42$) plant were selfed by bagging, and four spikes were backcrossed to both parents.

3.2.6. Morphological investigation

At maturity, the parents, F_1 hybrids produced by both procedures, and their amphiploids (C_1 to C_2) were studied for plant height, tiller number, leaf size (length and width), leaf area, spike length, dry weight, leaf/stem ratio, and fertility on a simgle plant basis. Pollen fertility was determined from pollen grains stained with 1.5% acetocarmine. Seed fertility was the proportion of seeds to the total number of florets.

3.2.7. Cytological observation

Somatic chromosome numbers were determined from metaphase spreads in root-tip cells which were treated in cold water at 2 ^{o}C for 24 h and then fixed in acetic acid (1) : ethylalcohol (3). Pollen mother cells (PMCs) were fixed in Carnoy's (ethylalcohol 6 : chloroform 3 : acetic acid 1) solution to observe chromosome behavior at metaphase I (MI), anaphase I (AI), and at the tetrad stages. Squash preparations for mitosis and meiosis were made in 1.5% acetocarmine solution.

3.3. Results

3.3.1. Production of F1 hybrids and amphiploids

The 70 pollinated florets of *E. canadensis* yielded 28 embryos (40%) of which 5 germinated on MS culture medium. Two mature plants were derived from the 28 embryos. They were identified as triploid hybrids. These two plants necrotized at the seedling and heading stages respectively. Twenty three embryos which did not germinate in embryo culture were transferred to MS callus culture medium. From them, one embryo produced white and compact calli (Fig. 3.1a). Four hybrid plants were regenerated via organogenesis from the calli that had been subcultured for three months (Fig. 3.1b). They grew vigorously to maturity.

Seven of the 480 clones which were treated with colchicine and DMSO had doubled sectors from which four amphiploids (C_1) were obtained. Nineteen C_2 plants were obtained from the 28 plump seeds of the C_1 plants which were open-pollinated without bagging. Two C_3 plants survived from twelve open-pollinated embryos from the C_2 amphiploids. One BC_1 plant was obtained from backcrossing of the C_2 hexaploid plant to *S. cereale*. No seed was obtained from backcrosses to *E. canadensis*.

3.3.2. Plant morphology and fertility

The morphological characteristics and dry weight of the parents, F_1 hybrids (both embryo-rescued and embryo-callus-regenerated), and C_1 and C_2 amphiploids are shown in Table 3.1. The data on the embryo-rescued hybrid that died at the heading stage were obtained prior to necrosis. Most of the characters of the callus-regenerated hybrids were intermediate between the parents but the hybrids produced more profuse tillers than the parents. As a result, dry weight of the hybrids was 64% and 335% higher than that of *E. canadensis* and *S. cereale*, respectively. However, the superiority of the hybrids over the parents in dry matter yield tended to decline during regrowth after clipping (data not shown). The leaf/stem ratio of the hybrids was significantly higher than that of the parents. There was no significant difference in morphology among the four callus-regenerated hybrids. Most of the hybrid pollen was empty and non-stainable. All of the hybrids were completely sterile.

Amphiploids (C_1 and C_2) showed a substantial loss in vigor compared to the F_1 hybrids and *E. canadensis* (Fig. 3.2a-e). C_1 amphiploid plants had smaller leaves than their parents and F_1 hybrids. Leaf area and dry weight of the C_1 plants were respectively 18% and 40% higher than those of *S. cereale* but 30% and 47% lower than those of *E. canadensis*. Spikes of the amphiploids were intermediate phenotype between the parents as shown in F_1 hybrids (Fig. 3.2f). The C_1 plants had an average of 56% pollen stainablility (Fig. 3.2g) and seed set averaged 4.6 seeds per spike. However, more than 90% of the seeds were shriveled and did not germinate. Twenty eight plump seeds, accounting for 9.3% of the total seeds, were intermediate in size between the parents (Fig. 3.2h). All germinated and 19 C_2 seedlings developed from them. Three early-maturing plants from these C_2 seedlings were examined for morphological characters and fertility. In comparison with C_1 plants, the C_2 plant ($2n=6x=42$) had increased leaf length and leaf width but reduced number of tillers (Fig. 3.2e). Among aneuploid progenies, vigor decreased with a decrease in chromosome number. One BC_1 plant generally resembled *S. cereale*, with low numbers of tillers and poor foliage.

Pollen stainability of the C_2 plants averaged 38% at the euploid level ($2n=42$) and 12% at the aneuploid level ($2n=40$). Only four of the other C_2 plants showed a low rate of pollen stainability: 1.7% ($2n=27$), 3.4% ($2n=27$), 8.3% ($2n=40$), and 12.7% ($2n=36$). Pollen from other plants was empty and nonstaining in acetocarmine. On the average, one seed per spike developed in the C_2 plant ($2n=42$) while the C_2 aneuploid was completely infertile. Most of these aneuploids lacked vigor and were inferior morphologically to the parents and F_1 hybrids (Fig. 3.2b-d). One BC_1 plant was completely sterile with 1.5% of pollen stainability.

3.3.3. Cytological behavior

The majority of the F_1 hybrid somatic cells were triploid ($2n=21$) and those of the C_1 amphiploids were hexaploid ($2n=42$) (Fig. 3.3a,b). However, among the nineteen C_2 plants, fourteen were examined cytologically and varied in somatic chromosome numbers from 26 to 42 (Table 3.3.). *Secale cereale* chromosomes were readily distinguished from *E. canadensis* chromosomes by their larger size (Fig. 3.3a-f). Chromosome constitution in the somatic cells of the amphiploid progeny could be identified on the basis of differences in the sizes of the chromosomes of *E. canadensis* and *S. cereale*. Hexaploid plants had 28 chromosomes of *E. canadensis* and 14 chromosomes of *S. cereale*. One of two monosomic plants (2n=41) lost one of the *E. canadensis* chromosomes and the other had lost one of the *S. cereale* chromosomes. A C_2 double monosomic plant (2n=40) lacked one chromosome of each parent, while a C_3 double monosomic plant (2n=40) lost two *E. canadensis* chromosomes. One C_2 plant (2n=39) lost three *S. cereale* chromosomes. The plants with 2n=36 and 37 chromosomes had 25 to 27 chromosomes from *E. canadensis* and 10 to 11 from *S. cereale* . Plants with 2n=26, 27, and 28 chromosomes had 13 to 17 from *E. canadensis* and 11 to 14 from *S. cereale* . One of the C_3 plants (2n=28) had 14 *E. canadensis* chromosomes and 14 *S. cereale* chromosmes. One BC_1 plant had 27 chromosomes (14 from *E. canadensis* and 13 from *S. cereale*).

Table 3.2 shows the frequencies of the different kinds of chromosome associations at metaphase I in the parents, F_1 hybrids (embryo-rescued and callus regenerated) from crosses between *E. canadensis* and *S. cereale*, and the amphiploids (C_1 to C_3) derived from these hybrids. *E. canadensis* and *S. cereale* genotypes used in this study showed respectively 13.9 and 6.9 bivalents per cell at MI. Ninety five percent of bivalents were ring-types in *E. canadensis* and 87% were ring-type bivalents in *S. cereale*. All hybrids showed low chromosome pairing ranging from 1.47 to 2.52 bivalents per meiocyte. The chromosomes of the callus-regenerated hybrids exhibited a higher frequency of the bivalents than those of the embryo-rescued hybrids. More than 85% of bivalents in all hybrids were loosely-or well-connected rod-types (Fig. 3.4a). Multivalents such as trivalents, quadrivalents, and pentavalents were rarely observed in the hybrids (Fig. 3.4a,b). The callus-regenerated hybrids showed quadrivalents in 1.2% of PMCs and pentavalents in 0.5% of PMCs. The most frequent chromosome associations at metaphase I in all hybrids at metaphase I were two bivalents and seventeen univalents. Most of the hybrid PMCs showed unequal chromosome disjunction at anaphase I (Fig. 3.4c) and some of them contained occasional laggards and bridges. Abnormal cytokinesis and micronuclei of variable numbers and sizes were occasionally observed at the tetrad stage (Fig. 3.4d).

Meiotic chromosome behavior of the amphiploids was characterized by the predominance of bivalents (Fig. 3.4e,f). Sixty seven percent of bivalents in the C_1 amphiploids was well-connected rod-types ranging from 1 to 15 per cell. In PMCs of the C_1 amphiploid plants, the most frequent chromosome configurations at metaphase I were 21 bivalents (34%) and 20 bivalents with 2 univalents (36%). Univalents averaged 2.20 per cell ranging from 0 to 14 and quadrivalents were observed in only two cells. Mean chiasma frequency per cell was 32.9. The chromosome distributions at anaphase I were predominately unequal (19:23 or 20:22) (Fig. 3.4h) and only 15% of the anaphase I cells showed equal chromosome disjunctions (21:21). Laggards and bridges were occasionally observed at anaphase I and micronuclei frequencies ranged from one to six in the tetrad stage (Fig. 3.4i).

The chromosome pairing of the C_2 amphiploids (2n=42) was almost the same as in the C_1 plants (Fig. 3.4g). However, the aneuploid plants with fewer chromosomes than the hexaploids had higher frequencies of univalents and trivalents than the hexaploid plants of C_1 and C_2 (Fig. 3.5a-e). The aneuploid plants with higher numbers of chromosomes (from 2n=36 to 2n=41) showed a lower frequency of univalents ranging from 3.73 to 6.12 per PMC. On the other hand, the aneuploid plants having lower number of chromosomes (2n=26, 2n=27, and 2n=28) showed an increased frequency of univalents (12.97 to 17.31). Multivalents were occasionally observed, more frequently in plants with higher chromosome numbers than in plants with lower chromosome numbers. Quadrivalents were found in only five plants among the twelve C_2 plants; two plants were 2n=28, one was 2n=36, one was 2n=37, and one was 2n=40. Table 3.3 shows meiotic chromosome associations at MI of the aneuploid progeny. Two monosomic plants(2n=41) frequently showed 19 II + 3 I and 18 II + 5 I, respectively and 18 II + 4 I was most frequent in the C_2 (37.8%) and C3 (26%) double monosomic plants (2n=40). The highest frequencies of chromosome configurations at MI in the C_2 plants of 2n=36 and 2n=37 were respectively 16 II + 4 I and 16 II + 5 I accounting for 31.3% and 28.8% of the total cells examined. showed The highest frequencies of chromosome configurations at metaphase I in the C_2 plants with 2n=26, 2n=27, and 2n=28 chromosomes were 8 II + 10 I (35.3%), 6 II + 15 I (24.6%) and 9 II + 9 I (22.0%), and 6 II + 16 I (26.7%) and 9 II + 10 I (25.2%), respectively. Four to seven bivalents and 14 to 18 univalents per PMC were commonly observed in the C_3 plants of 2n=28, of which 7 II + 14 I (20% of the total cells) indicates the genome formula of SHRR. Seven bivalents were observed in one backcross progeny (2n=4x-1=27, SHRR-1) of the amphiploid (SSHHRR) to *S. cereale* (RR). Chiasma frequency per cell ranged from 25.35 to 31.80 in the plants with higher numbers of chromosomes and from 6.63 to 9.58 in the plants of lower chromosome numbers in the C_2 and C_3 plants. The irregular chromosome behavior of the C_2 and C_3 plants at AI and in the quartet stage were of the same general nature as in the C_1 plants (Fig. 3.5f).

3.4. Discussion

3.4.1. Plant morphology

F_1 hybrids of *E. canadensis* with *S. cereale* (cv. ' Prolific') were first obtained through the conventional embryo rescue by Hang and Franckowiak (1984), but this study is the first report of regenerants derived from callus culture of *E. canadensis* x *S. cereale* (cv. 'Gazelle') hybrid embryo, and their colchicine-induced amphiploids. It is interesting that hybrid necrosis was not expressed in the regenerants while the embryo-rescued hybrids died of hybrid necrosis. This might be a result of genetic modification during the process of tissue culture. Hybrid necrosis which was found in some hybrids of wheat (Hermsen 1963) and *Agropyron* (Wang 1987a) was assumed to be controlled by complementary genes. In spite of hybrid vigor of the F_1, the reduction in tillering capacity and leaf size resulted in a decrease of dry matter yield in the amphiploids (C_1 and C_2). Although fertility was restored to some extent in the C_1 amphiploid plants, it declined in the C_2 plants. A substantial loss in vegetative vigor and a decline in fertility have been reported previously in high-ploidy-level (8x or 6x) amphiploids of *Elymus* x *Pseudoroegneria* hybrids (Asay and Dewey 1976; Dewey 1977) and *E. canadensis* x *Psathyrostachys juncea* hybrids (Park and Walton 1989). However, *E. canadensis* x *Pseudoroegneria libanotica* amphiploids (SSS'S'HH) showed some very desirable characteristics and cytological stability (Dewey 1974). The vigorous regrowth of the F_1 hybrids and amphiploids reported here indicates a perennial growth habit. However, the potential for increasing forage yield from enhanced regrowth and for improving forage quality was not studied.

3.4.2. Somatic chromosome complements

Hybrids from crosses between *E. canadensis* (SSHH) and *S. cereale* (RR) were allotriploids with genomes SHR and the allohexaploids possessed genomes SSHHRR. In aneuploid plants with 2n=36 to 2n=41 chromosomes, more *S. cereale* chromosomes tended to be eliminated than *E. canadensis* chromosomes. This might be due to the large sizes of *S. cereale* chromosomes as described by Shigenaga et al. (1971). These aneuploids with 36 to 41 chromosomes were probably the progeny of self-fertilizing or intercrossing among the C_1 or C_2 plants.

Plants with 26 to 28 chromosomes may be expected to result from outcrossing between the C_1 or C_2 plants and *S. cereale*. These results indicate that *E. canadensis* x *S. cereale* amphiploids can be cross-pollinated unlike largely self-fertilizing *E. canadensis*. Hill and Carnahan (1962) found a variety of plants with chromosome numbers of 14, 21, 28, 35, 42, 49, and 56 among the open pollinated progeny of *Lolium perenne* x *Festuca elatior* amphiploids (2n=6x=42). Dewey (1974) reported that about 10% of the C_2 plants open-pollinated from *E. canadensis* x *Pseudoroegneria libanotica* amphiploids (C_1) were outcrosses of C_1 plants with other species.

Meiotic irregularity followed by irregular formation of both female and male gametes of the parental amphiploids could be considered a plausible explanation for the aneuploid chromosome number of the progeny (Dewey 1980; Jan et al. 1986). Elimination of a portion of the genome constituents in advanced generations of interspecific or intergeneric amphiploids was observed in *Lolium multiflorum* x *Festuca elatior* amphiploids (Buckner et al. 1961), *Avena sativa* x *A. babarta* amphiploids (Nishiyama 1962), and *Elytrygia repens* x *Thinopyrum curvifolium* amphiploids (Dewey 1980).

3.4.3. Chromosome behavior during meiosis

The low chromosome pairing of the F_1 hybrids and the predominance of bivalents of the amphiploids indicate the distinctness among the genomes (S, H, and R). However, the occasional appearance of multivalents reflects a random intergenomic or intragenomic pairing, even though it is at a low frequency. A few reports have recently been published on the genomic relationships between the genomes SH (Asay et al., 1987), SR (Wang 1987b), HR (Gupta and Fedak 1985, 1987a, 1987b), and SHR (Hang and Franckowiak 1984). These studies indicate a remote phylogenetic relationship between these genomes. Limited intragenomic pairing was found in H and R genomes (Gupta and Fedak 1987a) and in S and

H genomes (Asay et al., 1987). If this is true, regardless of the genotypes used in hybridization, multivalents in this study may have resulted from the intragenomic pairing in H and R genomes or in S and H genomes.

The occurrence of trivalents and quadrivalents indicates a low potential for both intergenomic and intragenomic pairing and excludes the possible existence of a diploidizing gene similar to that described in hexaploid wheat (Riley and Chapman 1958) and tall fescue (Jauhar 1975). On the other hand, multivalent associations may be due to intergenomic pairing between chromosomes structurally rearranged by tissue culture and their intact homologous counterparts. This hypothesis would explain the higher frequency of chromosome pairing in the callus-regenerated hybrids than in the embryo-rescued hybrids. Induction of structural chromosome rearrangements accompanied by increasing chromosome associations such as bivalents and quadrivalents was reported in the regenerants from the cultured inflorescences or embryos of *Triticum* x *Hordeum* hybrids (Fedak and Grainger 1986) and *Hordeum vulgare* x *H. jubatum* hybrids (Orton 1980). Sterility in the *E. canadensis* x *S. cereale* amphiploids appears to be due to genetically unbalanced gametes resulting from heterogenetic pairing and meiotic irregularity.

In the present study, aneuploid plants which originated from the open-pollinated C_1 or C_2 plants showed variable chromosome configurations at metaphase I and abnormalities of chromosome behavior at anaphase I and later stages. Significant differences were noted in frequency of univalents between plants with 36 to 41 chromosomes (3.73-6.12/cell) and plants with 26 to 28 chromosomes

(12.97-17.31/cell). These trends reflect differences in the degree of genomic elimination among the progenies. There was 1.1 to 2.5 univalents per cell in the PMCs of the allohexaploids. The *E. canadensis* x *Psathyrostachys juncea* amphiploid (2n=6x=42, SSHHNN) averaged 5.9 univalents per PMC (Park and Walton 1989).

The majority of bivalents in the PMCs of aneuploid progenies could have resulted from preferential intragenomic pairing (S-S, H-H, or R-R). The similarity of pairing of 6 II + 15-16 I in plants with 28 chromosomes to the theoretical 7 II + 14 I of the allotetraploid (2n=4x=28, SHRR) supports the preposition that they originate from outcrosses of the amphiploid (SSHHRR) to *S. cereale* (RR). However, chromosome numbers of *E. canadensis* and *S. cereale* in each plant were more or less than expected from such outcrosses. Such deviations from that expected might be attributed to the different degrees and sites of chiasma formation. This also seemed to be associated with formation of unbalanced female gametes produced by the C_1 amphiploid plants and their random fertilization with balanced male gametes produced by *E. canadensis* or *S. cereale*.

The aneuploid plants showed frequent abnormalities of chromosome behavior such as unequal distribution of chromosomes, lagging chromosomes, chromatid bridges, and micronuclei at the anaphase, telophase, and tetrad stages. These meiotic irregularities resulted in extremely low pollen stainability and complete sterility. This study does not allow a determination of the associations between plant morphology and the modification of the genomes.

It is concluded that *E. canadensis* x *S. cereale* amphiploids (2n=6x=42, SSHHRR) are cytologically unstable and form an unequal number of female and male gametes, thereby giving rise to aneuploid progeny with variable numbers of chromosomes. Chromosome pairing in the aneuploid plants with low chromosome numbers (2n=26, 27, and 28), which frequently show univalents, could be improved if somatic chromosome numbers were doubled. However, the secondary amphiploids which are expected to have 2n=52, 54, and 56 chromosomes may be meiotically instable and sterile in the future generations, as shown in this study. Accordingly, this kind of allopolyploids combining *E. canadensis* with *S. cereale* may not be a suitable strategy for forage or cereal crop improvement. Such material may be used for forage or cereal breeding by introducing chromosome segments, but not whole chromosomes, of one species into another.

Although the performance of *E. canadensis* x *S. cereale* amphiploids in the greenhouse has fallen short of expectations, the opportunity for introgression between the two species by backcrossing to the parents and repeated somatic cell and tissue culture of the amphiploids still exists.

Table 3.1. Morphological characteristics of parents, F1 hybrids, and amphiploids (C1 and C2) from crosses between *E. canadensis* and *S. cereale*

Genotype	Nº of plants	Plant height (cm)	Nº of tillers	Leaf length (cm)	Leaf width (cm)	Spike length (cm)	Leaf area (cm2)	Dry weight (g)	Leaf/ stem ratio
E. canadensis	4	117.0	27.0	36.0	1.0	20.2	7.16	28.9	1:1.8
		#(7.62)	(4.08)	(1.43)	(0.08)	(0.71)	(0.36)	(2.52)	
S. cereale	4	99.8	14.3	25.0	1.3	9.2	4.21	10.9	1:2.1
		(7.52)	(4.50)	(2.50)	(0.08)	(0.42)	(0.28)	(1.19)	
Hybrid (embryo-rescued)	1	43.8	41.0	23.8	0.9	-	3.67	7.2	1:1.6
Hybrids (callus-regenerated)	4	115.6	86.5	25.8	1.1	15.7	5.29	47.4	1:3.2
		(5.12)	(3.11)	(2.12)	(0.08)	(1.69)	(0.62)	(2.15)	
Amphiploids (C1)	4	103.9	28.3	19.8	0.8	15.4	4.98	15.3	1:1.4
		(7.98)	(7.27)	(1.37)	(0.05)	(0.90)	(0.67)	(0.88)	
Amphiploids (C2) (2n=42)	2	94.9	7.0	26.6	1.0	19.3	3.52	5.5	1:1.5
		(18.8)	(2.83)	(7.07)	(0.14)	(0.28)	(0.48)	(0.99)	

Standard errors in parentheses

Table 3.2. Chromosome associations at MI of parents, F1 hybrids, and amphiploids (C1 and C2) from crosses between *E. canadensis* and *S. cereale*

Genotype	2n Genomes	Nº. of plants	Nº of cells	Mean (Range) number/cell						Chiasma frequency
				I	Rod II	Ring II	III	IV	V	
E. canadensis	28 SSHH	2	98	0.12 (0-4)	0.71 (0-3)	13.23 (11-13)				27.16
S. cereale	14 RR	2	113	0.23 (0-2)	0.87 (0-6)	6.01 (1-7)				12.03
Hybrid (embryo-rescued)	21 SHR	1	117	17.98 (11-21)	1.28 (0-4)	0.19 (0-2)	0.01 (0-1)	0.02 (0-1)		0.58
Hybrids (callus-regenerated)	21 SHR	4	417	16.56 (7-21)	1.77 (0-7)	0.28 (0-4)	0.06 (0-2)	0.02 (0-1)	0.02 (0-1)	0.95
Amphiploids (C1)	42 SSHHRR	2	119	2.20 (0-14)	6.61 5-20)	13.26 (1-15)		0.02 (0-1)		32.94
Amphiploids (C2)	42 SSHHRR	2	86	3.40 (0-14)	5.56 (2-10)	13.82 (6-19)	0.02 (0-1)			33.15

Table 3.3. Somatic chromosome constitution and meiotic chromosome associations at MI of aneuploid progeny from *E. canadensis* x *S. cereale* amphiploids (2n=6x=42)

Plants		Nº of chromosomes			Mean chromosome associations (Range)				Chiasma frequency	Nº of cells
		2n	*E. canadensis*	*S. cereale*	I	II	III	IV		
C2	1	41	27	14	5.40 (1-11)	17.80 (15-20)			31.80	50
	2	41	28	13	3.91 (1-7)	17.35 (17-20)	0.02 (0-1)		30.81	43
	3	40	27	13	3.73 (0-10)	18.02 (14-20)	0.07 (0-1)		31.11	164
	4	40	27	13	7.10 (0-20)	16.37 (1-17)	0.04 (0-1)	0.02 (0-1)	26.74	106
	5	39	28	11	4.25 (0-26)	17.22 (4-17)	0.10 (0-1)		28.26	68
	6	37	27	10	5.31 (0-11)	15.29 (7-18)	0.14 (0-1)	0.05 (0-1)	25.35	80
	7	36	25	11	3.25 (0-8)	16.29 (14-18)	0.02 (0-1)	0.02 (0-1)	28.92	83
	8	28	17	11	17.31 (10-24)	5.22 (1-9)	0.07 (0-1)	0.01 (0-1)	7.06	150
	9	28	15	13	16.03 (2-22)	5.92 (3-13)	0.03 (0-1)	0.01 (0-1)	7.76	103
	10	27	13	14	14.21 (9-19)	6.32 (3-10)	0.05 (0-1)		7.74	57
	11	27	15	12	16.53 (3-21)	5.04 (3-12)	0.13 (0-2)		6.63	150
	12	26	12	14	12.97 (4-18)	6.50 (3-11)	0.01 (0-1)		9.58	150
C3	1	40	26	14	6.12 (1-12)	16.76 (14-19)	0.12 (0-1)		29.06	50
	2	28	14	14	16.85 (7-24)	5.53 (2-9)	0.03 (0-1)		7.34	180

Fig. 3.1. Callus culture and plant regeneration of an *E. canadensis* x *S. cereale* hybrid embryo. **a.** hybrid embryo-derived callus (three month-old), **b.** plantlet regenerated from the callus.

b

Fig. 3.2. Plant morphology, spike, pollen, and seeds of *E. canadensis* x *S. cereale* hybrids. **a.** *E. canadensis*, **b.** *S. cereale*, **c.** F_1 hybrid, **d.** C_1 amphiploid, **e.** C_2 amphiploid (2n=6x=42), **f.** spike morphology of *E. canadensis*, F_1 hybrid, and *S. cereale* (from left), **g.** stainable pollen of C_1 amphiploid, **h.** two lows of seed of *E. canadensis*, amphiploid from C_1, and *S. cereale* (from left)

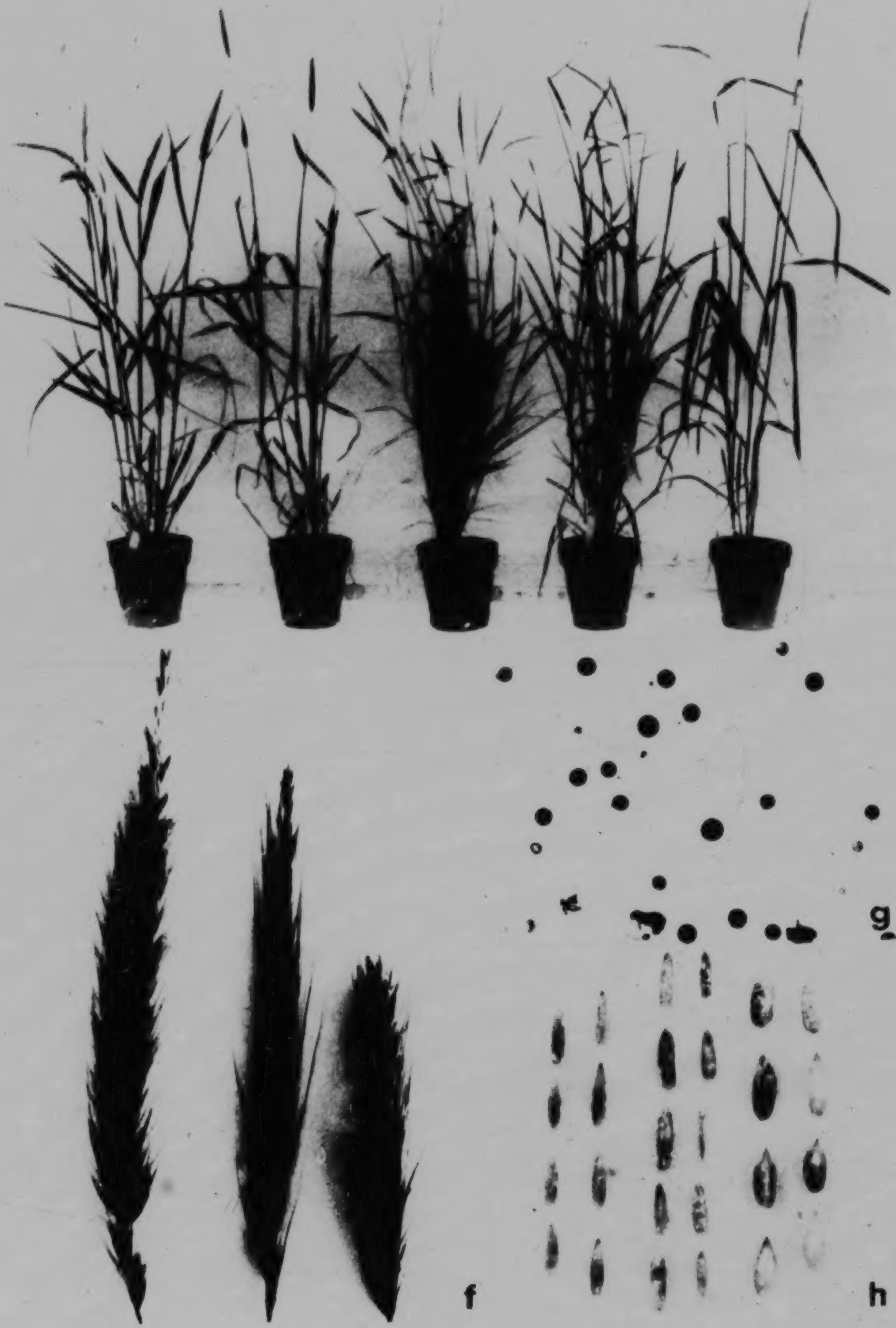
g
f
h

Fig. 3.3. Somatic chromosome complements of *E. canadensis* x *S. cereale* hybrids. **a.** F_1 hybrid (2n=3x=21), **b.** C_1 amphiploid (2n=6x=42), **c-f.** C_2 aneuploid progenies from C_1 amphiploid, **c.** 2n=41, **d.** 2n=39, **e.** 2n=36, **f.** 2n=27.

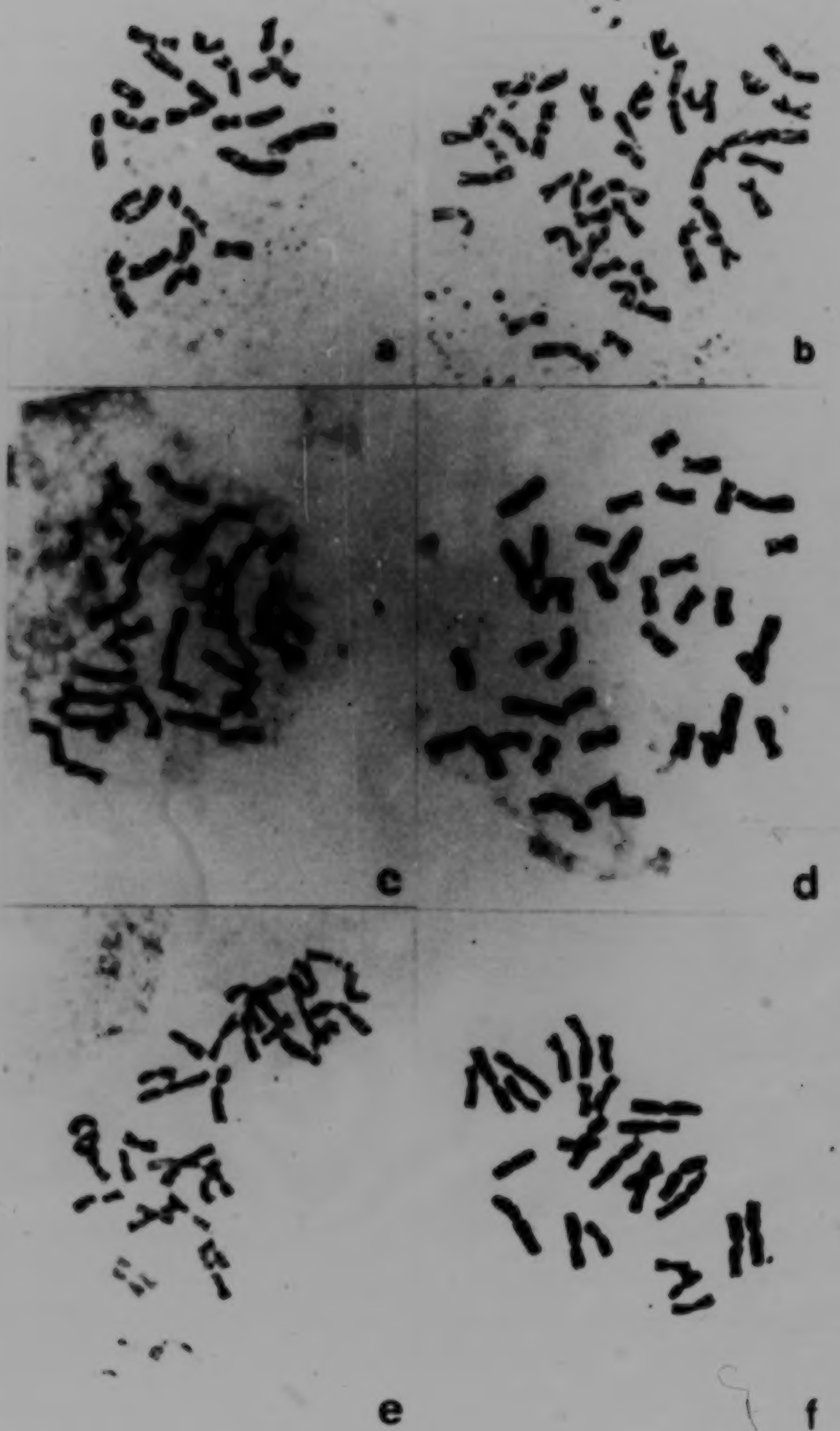
a
b
c
d
e
f

Fig. 3.4. Meiotic chromosome behavior of *E. canadensis* x *S. cereale* hybrids. **a.** 14 I + 2 II + 1 III (arrow) in a F_1 PMC at MI, **b.** 9 I + 2 II + 1 III + 1 V (arrow) in a F_1 PMC at MI, **c.** Unequal chromosome disjunction in a F_1 PMC at AI, **d.** Micronuclei in a F_1 PMC at the tetrad stage, **e.** 19 II + 1 IV (arrow) in a C_1 amphiploid PMC at MI, **f.** 8 I + 17 II in a C_1 amphiploid PMC at MI, **g.** 3 I + 18 II + 1 III in a C_2 amphiploid (2n=6x=42) PMC at MI, **h.** Unequal chromosome disjunction in a C_1 amphiploid PMC at AI, **i.** micronuclei in a C_2 amphiploid (2n=6x=42) at the tetrad stage.

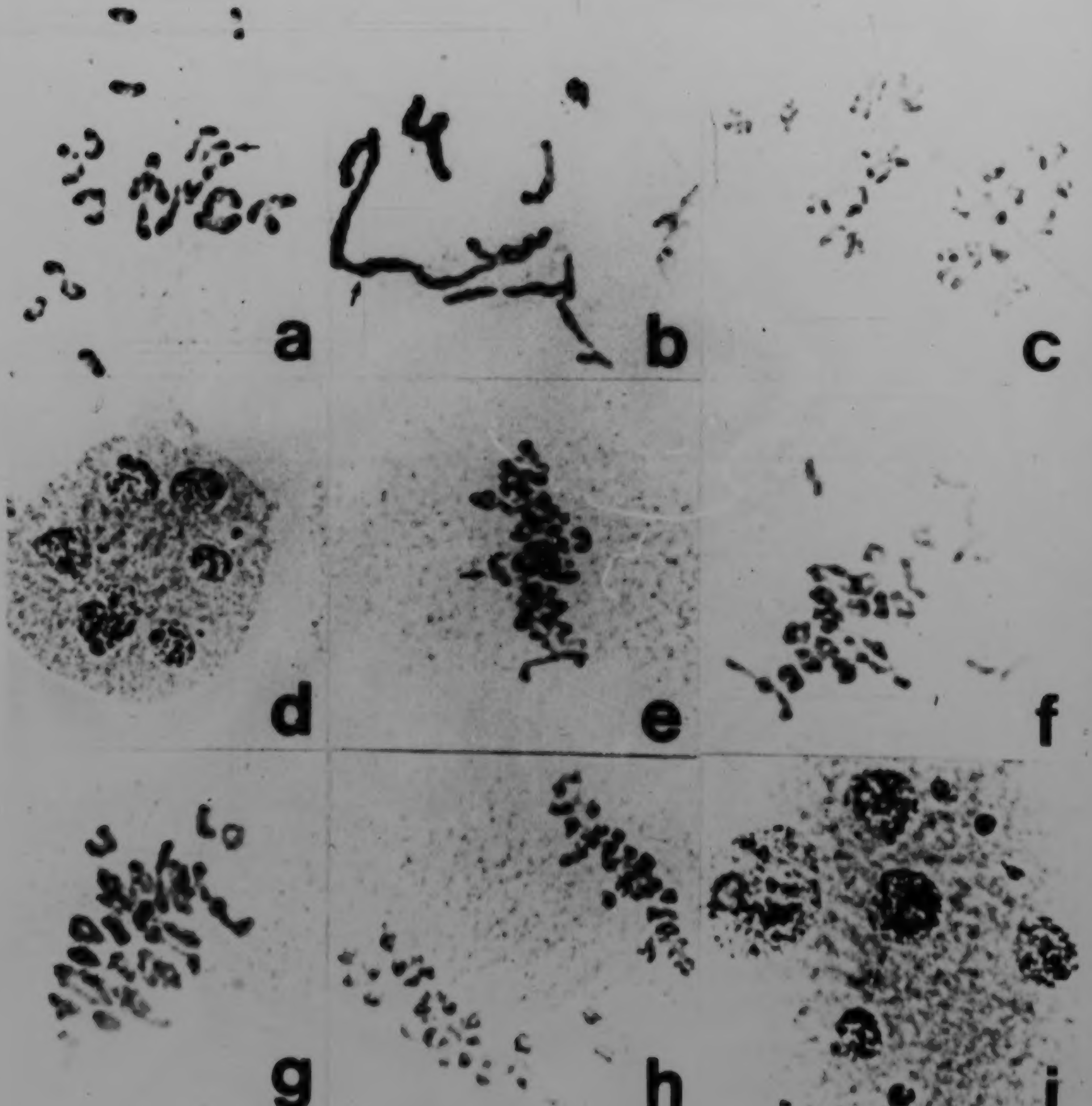
a
b
c
d
e
f
g
h
i

Fig. 3.5. Meiotic chromosome behavior of C_2 aneuploid progeny of *E. canadensis* x *S. cereale* amphiploid (C_1). **a.** multivalent formation (arrow) at diakinesis, **b.** 3 I + 17 II + 1 III in a PMC at MI (2n=40), **c.** 11 I + 11 II + 1 IV in a PMC at MI (2n=37), **d.** 16 I + 6 II in a PMC at MI (2n=28), **e.** 10 I + 8 II in a PMC at MI (2n=26), **f.** micronuclei in the BC_1 PMCs at the tetrad stage.

a
b
c
d
e
f

3.5. References

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4. EMBRYOGENESIS AND PLANT REGENERATION FROM TISSUE CULTURE OF CANADA WILDRYE, *Elymus canadensis* L.[1]

4.1. Introduction

Embryogenic or organogenic callus culture of the Gramineae has been readily obtained from immature embryos, immature inflorescences, and young leaves (Vasil, 1987). Plant regeneration from callus cultures of somatic tissues has been reported in many cereals and grasses (Lo et al., 1980; Vasil, 1982; Scowcroft, 1985). Depending on the somaclones, chromosome stability may or may not occur.

Canada wildrye (*Elymus canadensis* L.) is a North American native grass yielding a good quality forage in cool and moist regions (Walton, 1983). The species is a naturally self-fertilizing allotetraploid (2n=4x=28, SSHH) and has been crossed with cereal crops and other forage grasses for breeding disease resistant cereals and high yielding and good quality forages (Dewey, 1984; Mujeeb-kazi and Bernard, 1985).

Tissue culture techniques might be used either to improve Canada wildrye for use as a forage or to incorporate genetic material such as disease resistance into wheat. However, tissue culture-derived genetic variability or genome manipulation has not been previously reported in the genus *Elymus*. The present study was conducted to develop a procedure for embryogenesis and plant regeneration from immature embryos and inflorescences of Canada wildrye.

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[1] A version of this chapter has been published. Park & Walton 1989. Plant Cell Reports 8: 289-291.

4.2. Materials and methods

4.2.1. Explants

Plants of Canada wildrye (*Elymus canadensis* L.) accession 'Canada (PI-372539)' were grown in the greenhouse. From the self pollinated plants, 575 immature embryos ranging from less than 1 mm to 3 mm in length were excised 14 to 21 days after anthesis. Twenty one immature inflorescences were removed from their leaf sheath several days before heading. The young inflorescences which were 0.5 to 12 cm long were cut into 4 to 5 mm segments.

4.2.2. Callus culture

All immature embryos and inflorescence segments were sterilized in 70% ethyl alcohol for 1 min and subsequently in 4%-6% sodium hypochlorite for 10 min, and then rinsed three times in sterilized water. The explants were placed aseptically on MS (Murashige and Skoog 1962) agar medium containing 2 mg 2,4-D per litre. The test tubes (1.5 cm x 18 cm) with explants were kept under dark conditions for the first four weeks and then transferred to a culture chamber at 24±1 ^{o}C and 110 μE sec^{-1}cm^{2} fluorescent light. Four weeks after callus initiation, calli were subcultured monthly to fresh medium of the same type, to maintain embryogenic calli.

4.2.3. Plant regeneration

MS medium supplemented with 0.5 mg/l 2,4-D and 0.3 mg/l GA3 or hormone-free MS medium was used for plant regeneration. Plant regeneration was attempted monthly from two to six months after callus initiation at 24+1 ^{o}C and 110 uE sec^{-1}cm^{2} fluorescent light. The regenerated plantlets were transplanted to soil at the two to three leaf stage.

4.2.4. Histological and cytological observation

Histological preparations of somatic embryos were made by cryostats (IEC MINOT CUSTOM microtome) at -20 ^{o}C. Tissue -TekII was used for embedding tissue specimens. Frozen tissue specimens were prepared in thicknesses of 16 um. The number of chromosomes of a chlorophyll deficient regenerant was determined in root-tip cells pretreated with cold water at 2 ^{o}C for 24 h and fixed in acetic acid (1) : ethylalcohol (3). Tissue specimens of somatic embryos and root-tip cells for chromosome counting were stained with 1.5% acetocarmine.

4.3. Results and discussion

4.3.1. Callus production

Callus formation from immature embryos originated in the scutellar tissue within 5 to 10 days of inoculation. The percentage of embryos forming calli varied significantly with embryo size (Table 4.1). The frequency of germination (indicated by elongation of the plumules) and non-embryogenic callus formation were 4% and 12%, respectively. The optimum embryo size for maximum embryogenic callus formation was found to be 1.0 to 1.5 mm. This is similar to previous reports on embryo cultures of cereals (Shimada and Yamada, 1979; Nakamura and Keller, 1982; Rybczynski and Zdunczyk, 1986). Immature inflorescences produced calli at the floral primordia of the young florets within a week. Inflorescences which were 4 to 6 cm long exhibited a high frequency of embryogenesis (Table 4.1). Twenty three percent of inflorescence segments produced predominately watery and non-embryogenic calli, that were rapidly senescent even in subcultures.

Callus production in wheat and some temperate forage grasses has been shown to be successful with 0.5 to 1 cm long inflorescences (Ozias-Akins and Vasil, 1982; Maddock et al., 1983; Lo et al., 1980; Ahn et al., 1985). Inflorescence cultures of some intergeneric hybrids of the *Triticeae* formed calli rapidly with inflorescences that were 2.5 to 3.0 cm or 0.5 to 8 cm long (Chu et al., 1984; Fedak, 1985). In the present study, explants of the youngest inflorescences (less than 2 cm) did not produce callus, but explants of inflorescences larger than 8 cm formed calli. Callus formation was limited primarily to the premeiotic stage of the inflorescence, thereby indicating the effects of inflorescence developmental stages on callus production. The developmental stage of explants has been found to be a critical factor in the establishment of totipotent cultures (Vasil, 1987). The average frequency of embryogenesis in this study was 42% in cultures derived from immature embryos and 35% in cultures derived from immature inflorescences. The nature of embryogenic callus in the cultures of both explants was nodular and compact in appearance (Fig. 4.1a).

Embryoids of variable number, shape, and size appeared on the surface of calli (Fig. 4.1b, c).

Histological examination of the somatic embryoids revealed that the morphology and structure of the scutellum, coleoptile, and coleorhiza was similar to zygotic embryos (Fig. 4.1d, e). The shoot meristem was enclosed by the coleoptile and the root meristem by the coleorhiza as described for somatic embryos of *Triticum aestivum* (Ozias-Akins and Vasil, 1982), *Pennisetum americanum* (Vasil and Vasil, 1981), *Cynodon dactylon* (Ahn et al., 1985), and *Panicum maximum* (Lu and Vasil, 1981). Stereomicroscopic observations (Fig. 4.1f) and histological sections (Fig. 4.1g) showed shoots and roots developing from the somatic embryos.

4.3.2. Plant regeneration

Plant regeneration from immature embryo and inflorescence-derived cultures of *E. canadensis* was characterized by the development of single or multiple shoots with a variable number of roots and the development of roots without shoot regeneration. Table 4.2 shows thw number and percentage of calli exhibiting morphogenic responses on MS medium for plant regeneration during culture periods. Immature embryo-derived calli retained the ability to regenerate shoots and roots up to 120 days in culture. In spite of subculturing, the majority of four month-old embryogenic calli were so senescent that they were unable to differentiate shoots and roots. In inflorescence culture, however, calli maintained morphogenic capability for over 180 days. The rate of shoot and root regeneration was variable over culture periods but decreased markedly in the 6th month of culture. The trend might be due to the inconsistency in nature and extent of secondary and tertiary embryogenic callus which originated from primary embryogenic callus. No significant difference was found in the effects of 2,4-D and GA3 supplement on plant regeneration at the levels used here. In this study, a total of 357 plantlets were regenerated for 6-month culture.

Ten (2.8%) of the 357 plantlets regenerated were albino (Table 4.3. & Fig. 4.1h). The albino plantlets did not survive but one partially chlorophyll deficient plant grew to maturity and set seeds accounting for 16.2% of the total spikelets. This plant had white striped leaves on every stem and its somatic chromosome number was doubled ($2n=8x=56$) (Park and Walton, 1989). It is presumed that the chlorophyll deficiency was due to chromosome doubling which led to a doubled dosage of gene controlling chlorophyll deficiency. This hypothesis is supported by the occurrence of plants with slightly white-striped leaves in *E. canadensis* ($2n=4x=28$) (Park, unpublished). Accordingly, this kind of chlorophyll deficiency might be a result of external expression of pre-existing genetic factors after chromosome doubling. Appearance of albino plants regenerated from immature inflorescence-derived calli has been described in *Pennisetum americanum* (Swedlund and vasil, 1985), *Echinochloa oryzicola* (Takahashi et al., 1984), *Bromus inermis, Alopecurus arundinaceus, Agropyron cristatum,* and *Stipa viridula* (Lo et al., 1980). Among them, doubling of chromosome numbers was found only in *Echinochloa oryzicola* and *Pennisetum americanum.* Finally, only 192 regenerants grew to maturity because many young plantlets had poor root systems at the time of transplanting.

In conclusion, this study establishes the optimum size of explants for embryogenesis and plant regeneration from culture of immature embryos and inflorescences of *E. canadensis*. This study was conducted by supplementing only one or two hormones (2,4-D and GA3) to a single (MS) culture medium. Accordingly, the culture protocol should be modified to enhance yields of calli and regenerants from the explants with variable developmental stages.

Table 4.1. Frequency of embryogenesis from immature embryo and inflorescence culture of *E. canadensis*

Explant sources	Explant sizes (mm)	Nº of explants inoculated	Nº (%) of explants producing embryogenic calli
Immature embryos	less than 1.0	140	52 (36.6)b*
	1.0 - 1.5	82	50 (61.0)a
	1.5 - 2.0	244	97 (39.8)b
	2.0 - 3.0	109	42 (38.5)b
Immature inflorescence	5 - 20	25	0 (0)b
	20 - 40	50	19 (38.0)b
	40 - 60	46	26 (56.5)a
	60 - 80	39	18 (46.2)a
	80 - 120	40	7 (17.5)b

* Values followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test, within explant source.

Table 4.2. Numbers and percent (in parentheses) of calli showing morphogenic response from immature embryo and inflorescence culture of *E. canadensis*

Explant sources	Culture periods (days)	Nº of calli inoculated	Nº (%) of calli forming shoots + roots	Nº (%) of calli forming roots
Immature embryos	60	75	18 (24.0)a*	23 (30.7)a
	90	87	23 (26.4)a	20 (23.0)a
	120	79	25 (31.6)a	32 (40.5)a
Immature inflorescences	60	52	29 (55.8)a	11 (21.2)a
	90	49	23 (46.9)ab	12 (24.5)a
	120	161	83 (51.6)a	44 (27.3)a
	150	215	116 (54.0)a	70 (32.6)a
	180	110	40 (36.4)b	39 (35.5)a

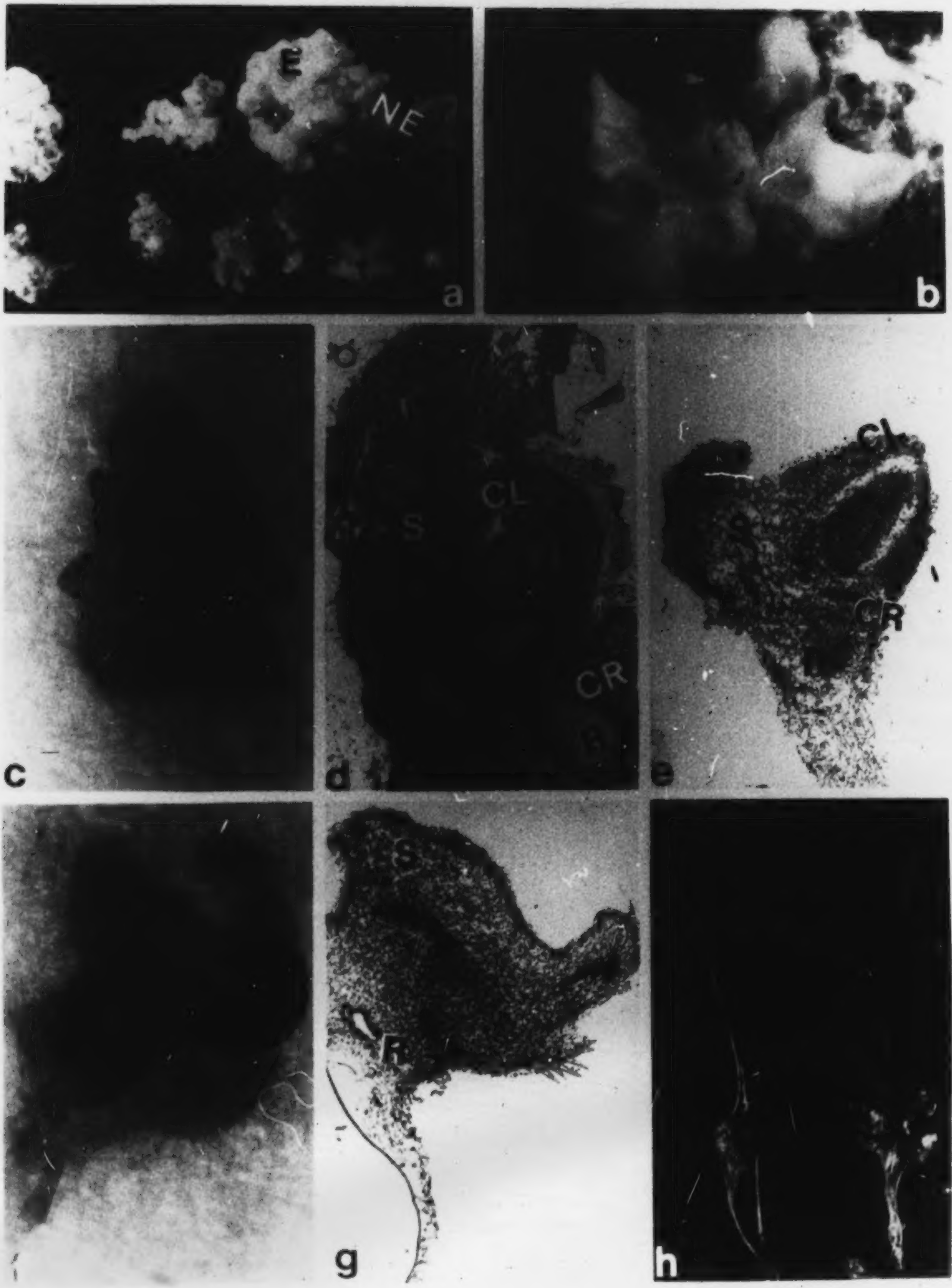
* Values followed by the same latter are not significantly different at the 5% level according to Duncan's multiple range test, within explant source.

Table 4.3. Frequency of chlorophyll deficient (albino) among *E. canadensis* regenerants

Explant sources	Culture periods (days)	Nº of regenerants	Nº (%) of chlorophyll deficients (albinos)	
			white	light violet
Immature embryos	60	18		2 (11.1)
	120	25	2 (8.0)	
Immature inflorescences	150	116	2 (1.7)	3 (2.6)
	180	40	1 (2.5)#	

# One chlorophyll-deficient plant with white-striped leaves survived.

Fig. 4.1. Callus culture and plant regeneration of *E. canadensis*. **a.** embryogenic callus (E) and nonembryogenic callus (N), **b-c.** somatic embryos, **d.** longisection of a somatic embryo (b) (CL: coleoptile, CR: coleorrhiza, R: radicle, S: scutellum), **e.** longisection of a torpedo-typed embryo (c), **f.** shoot and root developing from somatic embryo, **g.** longisection of shoot and root (f), **h.** green (right) and albino (left) plantlets regenerated.


E
NE
a
b
c
CL
S
CR
d
CL
CR
e
S
R
g
h

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5. MORPHOLOGY AND CYTOLOGY OF A TISSUE-CULTURE-DERIVED OCTOPLOID OF *Elymus canadensis* AND ITS SELFED AND BACKCROSSED PROGENY[1]

5.1. Introduction

The *Elymus* species, which occur in nature as polyploids, form the largest genus in the Triticeae. About 75% of the species are allotetraploids (SSHH) and have a genome derived from *Pseudoroegneria* (SS) and a genome from *Critesion* (=*Hordeum* HH) (Dewey, 1984a). These species have been used as cool season forage grasses (Walton, 1983) and are regarded as being of value in breeding disease resistant wheat (Sharma et al., 1984; Mujeeb-kazi and Bernard, 1985).

Genomic manipulation, involving the addition or replacement of entire genomes, has been used to studies of evolutionary patterns in Triticeae and to aid alien gene transfers for agronomic gains. Hexaploids produced by adding two genomes to the tetraploid *Elymus* chromosome complement (SSHH) are of the following type:

(1) segmental autoallohexaploids of the type SSS'S'HH or SSHHH'H' (Dewey, 1984a)

(2) natural hexaploids of the types SSSHHH (Sadasivaiah and Weijer, 1981), SSHHYY (Dewey, 1980), and SSYYXX (Jensen et al., 1986)

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[1] A version of this chapter has been partly published, Park & Walton 1989. Plant Breeding 108: 208-214, and partly submitted for publication, Park & Walton 1989. Genome.

(3) interspecific hexaploid hybrids (SS'HH'YY') from crosses between two natural hexaploids (Sakamoto and Muramatsu 1966).

(4) induced hexaploids (SSSHHH) from a cross between induced octoploids and tetraploids (Aung and Walton 1987).

(5) interspecific and interploidy hexaploid hybrids [(SSS'HHH' from a cross, 8X ('A' species, SSSSHHHH) x 4X ('B' species, S'S'H'H')] (Aung and Walton 1989).

Canada wildrye (*Elymus canadensis* L.) exists naturally as a tetraploid in North America. This species has been used for forage and has been hybridized with other Triticeae grasses and cereals to develop new germplasm more adaptable to unfavorable environments and diseases, and also to analyze genome relationships with other species (Dewey 1984a). However, the nature of the S and H genomes and the genetic variability of the species has not been extensively studied.

Apart from the potential of induced hexaploid and octoploid *Elymus* for improved agronomic gains, hexaploids would be a good source of pentaploids which may produce trisomics and tetrasomics through backcrossing to a tetraploid as a step in the process of transferring single valuable characteristics. In addition, various kinds of trisomics can be employed to study the effect of duplication of a whole chromosome, a chromosome arm or part of a chromosome arm on the morphology, anatomy, and physiology of the organism. This can throw light on the basic nature of the genome of the species (Khush 1973).

In our laboratory, we are investigating the nature of genomes of *Elymus* using *E. trachycaulus* and *E. canadensis* (Aung and Walton

1987, 1989; Park and Walton 1989a; Kumar and Walton, unpublished). This study was undertaken to morphologically and cytologically characterize an octoploid obtained from tissue culture of Canada wildrye and its selfed and backcrossed progeny, including hexaploids, pentaploids, tetrasomics, and trisomics.

5.2. Materials and methods

5.2.1. Plant materials and embryo rescue

A partially chlorophyll-deficient octoploid plant of *Elymus canadensis*, accession 'Canada (PI-372539)' was previously obtained from immature inflorescence culture (Park and Walton 1989c). Some spikes of the octoploid plant were self-fertilized and others were emasculated and pollinated by the two accessions 'Canada (PI-372539)' and 'Montana (PI-232249)' of tetraploid *E. canadensis*. Among the progeny, a hexaploid plant from a cross between two different ploidy levels (8X x 4X) of the same accession 'Canada' was emasculated and backcrossed to the tetraploid plant. Embryos were rescued as described in 3.2.3.

5.2.2. Morphological and cytological investigation

Morphological and cytological studies are those described in 3.2.6 and 3.2.7, respectively.

5.3. Results

5.3.1. Production of selfed progeny

One tissue culture-derived octoploid was fertile and set 60 seeds on three spikes. From the 60 seeds, 43 germinated and 38 plants grew to maturity. The 38 selfed progeny consisted of 35 octoploids (2n=8x=56), 2 aneuploids (2n=8x-2=54), and 1 hexaploid (2n=6x=42).

5.3.2. Production of backcross progeny

The results of crossing two different tetraploid accessions ('Canada' and 'Montana') with octoploid and hexaploid 'Canada' are shown in Table 5.1. None of the plants from the cross between hexaploid 'Canada' and tetraploid 'Montana' obtained. The crossability (no. of plants survived/no. of florets pollinated) was 11 to 12% in the crosses of the octoploid with both accessions of the tetraploid and 8% in the hexaploid x tetraploid cross.

5.3.3. Morphological characteristics

The octoploid regenerated from callus culture of the immature inflorescence showed substantial increase over the tetraploids in leaf size and stem thickness. Leaves on every stem were white striped (Fig. 5.1a) and 50% larger than those of the tetraploids (Fig. 5.1b). Plant height and tiller number of the octoploid regenerant averaged 74.3 cm and 22.3, respectively, and dry matter yield was 26% higher than the tetraploids (Table 5.2). The plant was relatively fertile and produced

20 seeds per spike.

The genotypes of the selfed octoploid progeny were extremely variable in morphology, fertility, and dry matter yield. With the exception of plant height, all characters of the 35 genotypes had average values lower than the parental octoploid (octoploid regenerant). Only one plant was superior to the parental octoploid in all of the characters investigated. Fertility of the selfed octoploids varied from 0 to 36.1% and averaged 15.9%. Two aneuploid plants having two chromosomes less than the octoploid had extremely poor vigor. Seed fertility of the plants averaged 7.0%.

One hexaploid plant was more vigorous than the octoploids and the tetraploids (Fig. 5.1d). The plant showed moderate plant height and tillering capacity but larger leaves than the octoploids and tetraploids. Leaf dry weight was twice the stem dry weight (leaf/setm ratio 2.0) and total dry weight was 68% to 52% higher than that of the octoploids and the tetraploids, respectively. Fertility of the hexaploid was only 7.4%.

Progenies from the octoploid x tetraploid cross were vigorous and slightly different in morphology from plant to plant. Their somatic chromosome numbers ranged from 39 to 43. Five morphological characteristics such as plant height, tiller number, leaf width, leaf length, and spike length differed slightly within the progenies of 8X x 4X crosses, regardless of which tetraploid accession was involved (Table 5.3). A plant with 42 chromosomes from the 8X ('Canada') x 4X ('Canada') cross had the tallest stems and longest leaves (Fig. 5.1g). Pollen stainability varied from plant to plant. Pollen of the hexaploid plants stained better than aneuploid progenies from crosses of 8X x

4X. Hexaploid plants had limited seed set (1 to 3%).

One hexaploid plant obtained from a cross involving accession 'Canada' in both parents was backcrossed to the same tetraploid accession. Six progenies survived and were variable in chromosome number: one was pentaploid (Fig. 5.1h) and others were aneuploids, including one tetrasomic plant and two trisomic plants. The aneuploid plants with 2n=29, 30, 32, 34, and 35 grew slowly and were less vigorous than those with 2n=39, 40, and 43 (Table 5.3). Compared to other progenies of the 6X x 4X cross, tetrasomics and trisomics had reduced leaf and spike sizes (Fig. 5.1i,j) but they had stainable pollen and set some seeds (17 to 19% seed fertility). Two trisomic plants were morphologically indistinguishable.

5.3.4. Cytological behavior

The somatic chromosome numbers of individual plants was identified in 30-50 cells at the metaphase or anaphase stage (Fig. 5.2). Meiotic chromosome behavior of the individual plants representing each ploidy level was observed in 30 or more microsporocytes (Table 5.4). The tetraploid formed 14 ring bivalents at metaphase I and chromosome disjunction at anaphase I and II was normal. The octoploids, both regenerated and selfed, showed several multivalents, bivalents, and univalents at diakinesis, metaphase I, and metaphase II (Fig. 5.3a, b, c). Ring-type quadrivalents were the most common multivalent association observed. The mean chromosome configurations of the octoploid regenerant and one selfed octoploid at metaphase I were 0.97 I + 21.23 II + 0.83 III + 2.57 IV and 1.25 I +

19.66 II + 1.06 III + 3.06 IV, respectively. Unequal disjunction of chromosomes, laggards, and chromatid bridges were commonly observed at anaphase I and II in the octoploids. Equal segregation (28:28) occurred in 38% of the meiocytes at anaphase I in the octoploid regenerant. The most frequent chromosome segregation at anaphase I was 27:29 (35-47%) in the selfed octoploid plants. The aneuploid PMCs showed a higher degree of meiotic irregularity than other ploidy levels. An average of 4.69 I + 16.54 II + 0.85 III + 2.23 IV per cell was observed, with a range of 0 to 13 univalents. Meiotic irregularity of the aneuploids had the same general features as in the octoploids. Chromosome pairing in the hexaploid PMCs was characterized by the predominance of trivalents and bivalents (Fig. 5.3d). The mean frequencies of chromosome associations in the hexaploid at metaphase I were 3.14 I + 8.25 II + 8.34 III + 0.41 IV. Trivalent frequency varied from 0 to 9 per cell. More than 90% of anaphase I cells exhibited unequal chromosome segregation (predominantly 19:23) and occasionally contained from 1 to 7 laggards(Fig. 5.3e). Chromatid bridges were observed at both anaphase I and anaphase II (Fig. 5.3f).

Somatic chromosome numbers of all backcross progenies were consistent in 30 cells examined (Fig. 5.2). Meiotic pairing at the metaphase I varied slightly within progenies of 8X x 4X crosses (Table 5.5). Slight differences in frequencies of univalents, bivalents, and trivalents were found between progenies from the 8x ('Canada') x 4X ('Canada') cross and progenies from 8X ('Canada') x 4X ('Montana'). Wide variation in pairing mode was observed in the hexaploid plants. The mean frequencies of the different chromosome associations in the hexaploid PMCs at MI were 2-3 I + 14-17 II + 2-4 III + 0.1 IV (Fig. 5.4a). In both crosses, aneuploid plants having 43 or 40 chromosomes averaged 3 to 4 I, 14 to 17 II, and 2 to 3 III per cell. A pentaploid plant from the 6X x 4X cross showed mean chromosome associations of 2 I, 15 II, and 1 III per cell (Fig. 5.4b). In one tetrasomic plant, 1 I + 13 II + 1 III was observed in 33% of the meiocytes (Fig. 5.4c). About 17% the meiocytes showed complete chromosome pairing (15 II). Chromosome associations of 13 II + 1 III were observed in 38% of the meiocytes of one trisomic plant and 20% of the meiocytes of another one (Table 5.6 & Fig. 5.4d). Most of the PMCs in two trisomic plants (2n=4x+1=29) formed 1 I and 14 II per cell (Fig. 5.4e). Regardless of the accessions involved in crosses and the chromosome numbers of the progenies, bivalents formed predominantly ring-type configurations. Quadrivalents were occasionally observed in all of the progenies except in a pentaploid plant.

In the backcross-derived hexaploid plants, unequal segregations (20:22 to 16:26) occurred in the majority of the meiocytes at anaphase I. The most frequent chromosome segregation was 19:23, accounting for 41% of the total distributions at anaphase I. Eighty five percent of the meiocytes had 1 to 8 lagging chromosomes at anaphase I (Fig. 5.4f). From 1 to 6 micronuclei of variable sizes were observed in 41% of the quartets. Frequencies of lagging chromosomes and micronuclei in aneuploid progenies from 8X x 4X crosses were 90-92% and 57-88%, respectively. I a pentaploid plant, 53% of the meiocytes had lagging chromosomes at anaphase I and 86% had micronuclei at the quartet stage. Frequencies (and range) of lagging chromosomes at anaphase I were 51% (1 to 4) of the meiocytes in tetrasomics and

21% (1 to 3) in trisomics (Fig. 5.4g). Frequencies (and range) of micronuclei in quartet were 55% (1 to 8) in a tetrasomic plant and 25% (1 to 8) in trisomic plants (Fig. 5.4h).

5.4. Discussion

5.4.1. Plant morphology

Previous reports have made of polyploids induced in tissue culture (D'Amato 1977; Kasperbauer et al. 1979; Swedlund and Vasil 1985; Gaponenko et al. 1988). Pre-existing chromosome variation, nuclear fragmentation, endoreduplication or endomitosis, and abnormality of mitosis were assumed to influence the variation in chromosome number during tissue culture (Evans et al. 1984). However, morphological and cytological information has rarely been presented for the tissue culture-induced polyploids and their progeny.

The octoploid regenerant of Canada wildrye which was found in this study showed general features of polyploids such as a reduction in tiller numbers, gigas leaves and stems, meiotic irregularity, and low fertility. Of 38 plants obtained from the self-fertilized octoploid regenerant, 35 (92%) were octoploid. The progeny derived from self-fertilization of the octoploid varied in all of the characters examined, probably resulting from gene recombination. Despite reduction in tillering capacity, an increase in leaf size resulted in a higher dry matter yield in some octoploids and a hexaploid. In this study, increased morphological vigor in hexaploid plants which were obtained both from selfing (probably intercrossing) and from 8X x 4X crosses seemed to originate from genic causes. This is similar to that described for the induced hexaploid Slender wheatgrass (*Elymus trachycaulus*) which was obtained by backcrossing a colchicine-induced octoploid to a tetraploid (Aung and Walton 1987). In both cases, morphological superiority was assumed to be due to a triple dosage of genes in the genomic constituents of the double triploid (SSSHHH). The similar phenotypes of progenies from 8X ('Canada') x 4X ('Canada') and those from 8X ('Canada') x 4X ('Montana') indicate that the two accessions of *E. canadensis* are very similar genotypes. Among the progenies from 8X x 4X crosses, aneuploid plants (2n=6x-2=40) were morphologically similar to the hexaploid plant. This might result from the performance of duplicated genes on other chromosomes on behalf of functions carried out by the missing genes (Kuspira 1986). This study reports on trisomics and tetrasomics of *E. canadensis* for the first time. The different trisomics of *E. canadensis* are morphologically indistinguishable. The same has been found to be the case in other polyploid species, i.e. *Triticum aestivum* and *Avena sativa* (Khush 1973).

5.4.2. Cytological behavior

Most of the chromosomes in octoploid paired autosyndetically in the form of bivalents or quadrivalents. Predominance of bivalents in the metaphase I in the parental octoploid and tetraploid *E. canadensis* was previously found and indicated intragenomic pairing (S-S and H-H). Kumar and Walton (unpublished) suggested that the predominance of bivalents in the amphiploids (8X) of *E. trachycaulus* and *E. canadensis* hybrids as well as in the octoploid plants of parental *Elymus* species might be controlled by a polygenic diploidizing mechanism. The diploidizing system affects homologous and homoeologous chromosomes by reducing the multivalent frequency during meiosis in the autopolyploid plants (Wang 1989). Pentavalents and hexavalents were not observed in these octoploid PMCs. A natural octoploid Great Basin wildrye (*Leymus cinereus* JJJJNNNN) contained a few pentavalents and hexavalents in PMCs (Dewey 1966).

On the basis of the frequent occurrence of trivalents, hexaploid plants obtained in this study are regarded to be genomically double triploids (SSSHHH). Doubled triploidy (SSSHHH) in the *Elymus* genus was reported in a natural hexaploid Northern wheatgrass (*Elymus lanceolatus*) (Sadasivaiah and Weijer 1981) and for the induced hexaploid Slender wheatgrass (Aung and Walton 1987). However, bivalents and univalents were commonly observed in the PMCs of the hexaploid *E. canadensis* plants. This might be due to the failure of chiasma formation at pachytene and chiasma terminalization at the later stages of meiosis. Tetrasomics and trisomics obtained from a 6X x 4X cross also indicate that the hexaploid female parent is genomically a double triploid (SSSHHH), because triploids are the best and most dependable sources of trisomics in diploid species (Khush 1973). Moreover, the cytologically diploid-like nature of the octoploid genomes may lead to relatively regular meiotic division and subsequently form, to some extent, balanced and functional gametes. The predominant bivalent formation at metaphase I, relatively equal chromosome disjunction at anaphase I, and fairly stainable pollen and seed set in the parental octoploid plants also support this notion. For this reason, the doubled triploid genomes (SSSHHH) could be result of backcrossing an octoploid to a tetraploid. However, it is unknown how the hexaploid originated from selfing an octoploid regenerant. It may have resulted from fertilization of a reduced SSHH octoploid gamete (female) with a reduced SH tetraploid gamete (male). If this is indeed the mechanism involved in the origin of the hexaploid, one would expect the hexaploid to show the same pairing tendencies as other hexaploids obtained by backcrossing the octoploid regenerant to the tetraploid. However, there is a substantial difference in the frequency of trivalents between the hexaploid obtained from octoploid regenerant and the hexaploid produced from backcrosses, leaving the question unanswered. A pentaploid consists of five genomes (SSSHH or SSHHH), but, it is not known which genome is in triplicate. Fifteeen bivalents in 17% of the meiocytes in a tetrasomic plant indicates that two extra chromosomes are basically homologous and this leads to fairly stainable pollen and 19% seed set. The most frequent chromosome association was 1 I + 14 II rather than 13 II + 1 III in the trisomics at metaphase I. This might also indicate a high potential for failure of chiasma formation. The failure of chiasma formation may increase the fequency of univalents in these genomes, leading to limited recombination and sterility. Genetic variability of *E. canadensis* and gene introgression from this species into other species may therefore be limited. Wang (1984) discovered a desynaptic variant in the diploid *Critesion violaceum* in which low chiasma frequency at metaphase I might be controlled by the dosage effect of the desynaptic gene, which was probably recessive. However, it is not clear if the low frequency of trivalents in these trisomics is controlled by a recessive desynaptic gene. Chiasma frequency is closely related to chromosome size, i.e. the longer chromosomes have a higher chiasma frequency than shorter ones (Khush 1973). The differences in chromosome sizes and chiasma frequency between the S genome and H genome of *Elymus* species have not been determined with certainty because the donor species of the H genome is not known.

The extra chromosome in the two trisomic plants was not identified by karyotype analyses. However, the differences in chromosome pairing configurations between the trisomic plants may indicate that they are different trisomics. Variable frequencies of the chromosome associations have previously been found in different trisomics of tomato (Rick and Barton 1954), barley (Tsuchiya 1963), and rice (Watanabe and Koga 1975). It may thus be possible to produce a complete series of trisomics of this species provided the same cross (6X x 4X) is repeated extensively.

Generally, chromosomal irregularity, resultant reduction in fertility, and lack of vigor are known to make the induced polyploids at high ploidy levels unfavorable for agronomic use. For these reasons, Asay and Dewey (1976) believed that producing autoallooctoploids in *Elymus* was a negative strategy for forage breeding. However, Aung and Walton (1987) demonstrated that the gigas and partially fertile hexaploid Slender wheatgrass, which might be due to genomic triploidy, could be vegetatively propagated for practical use and would be useful in producing primary trisomic and tetrasomic lines as genetic stocks for gene mapping. In fact, this study indicates that the production of a complete trisomic series of *E. canadensis* by crossing hexaploids with tetraploids and by selfing of trisomics and tetrasomics would provide substantial information on the nature of the genomes of this species as well as on the location of important genes on the chromosomes. The octoploids and hexaploids of *E. canadensis* may also be used in crosses with diploid Triticeae grasses to produce alien chromosome addition or substitution lines for forage or cereal crop improvement.

Consequently, this study confirmed genome modification in tissue culture and the potential of octoploids and hexaploids as raw polyploids for forage or cereal breeding and genetic studies of Canada wildrye.

Table 5.1. Frequencies of embryos and plants obtained from the crosses, 8X x 4X and 6X x 4X, and chromosome numbers of progenies

Cross#	Nº of florets pollinated	Nº of embryos cultured	Nº of plants survived	Nº of chromosomes (2n)##
8X ECC x 4X ECC	25	8	3	42, 40, 39
8X ECC x 4X ECM	45	15	5	43, 42(2), 40(2)
6X ECC x 4X ECC	72	12	6	35, 34, 32, 30, 29(2)
6X ECC x 4X ECM	28			

# ECC = *Elymus canadensis* accession 'Canada (PI-372539)'
ECM= *Elymus canadensis* accession 'Montana (PI-232249)'
## Nº of plants in parentheses

Table 5.2. Morphological characteristics and fertility of four ploidy levels of *E. canadensis*

Ploidy level	2n	Plant height (cm)	Nº of tillers	Leaf length (cm)	Leaf width (mm)	Dry weight (g)	Leaf/ stem ratio	Seed fertility (%)
Octoploid regenerant	56	67.1	35	23.2	88	20.4	1.83	28.0
Octoploids selfed#	56	74.3	22.3	22.5	84	14.6	1.68	15.9
Hexaploid from 8X	42	76.2	44	22.0	102	24.6	2.0	7.4
Aneuploids#	54	49.4	33.0	19.7	57	8.5	2.16	7.0
Tetraploids#	28	82.3	50.0	15.5	87	16.2	1.69	39.6

Values are mean measurements for 35 octoploids, 2 aneuploids, and 5 tetraploids, respectively.

Table 5.3. Morphological characteristics and fertility of progenies obtained from the crosses, 8X x 4X and 6X x 4X, in *E. canadensis*

Cross	Nº of chromo-somes(2n)	Nº of plants	Plant height (cm)	Nº of tillers	Leaf length (cm)	Leaf width (cm)	Spike length (cm)	Pollen stainability (%)	Seed fertility (%)
8X ECC x 4X ECC	42	1	97.5	32	30.2	1.11	17.1	23.2	2.9
	40	1	90.5	33	26.5	0.9	17.4	14.8	0.0
	39	1	82.3	30	28.0	0.9	17.2	10.4	0.0
8X ECC x 4X ECM	43	1	84.8	34	27.5	0.88	18.0	6.9	0.0
	42	2#	86.9	34	26.9	0.9	17.6	26.2	1.3
	40	2#	82.9	25	25.7	0.96	17.7	22.5	0.0
6X ECC x 4X ECC	35	1	75.4	35	24.8	0.88	17.8	25.4	0.0
	34	1	63.8	37	27.5	0.75	15.8	13.2	0.0
	32	1	70.2	31	25.5	0.75	17.0	18.7	0.0
	30	1	60.8	38	22.0	0.59	16.2	64.5	19.1
	29	2#	68.3	35	24.7	0.71	14.8	55.4	17.4

Values are mean of two plants.

Table 5.4. Mean number of chromosome configurations observed per microsporocyte at MI in octoploids and hexaploids of *Elymus* species and hybrid from cross between *Elymus trachycaulus* and *Elymus canadensis*

Species and hybrid	Genomes	Mean chromosome configurations (Range)						Sources
		I	II	III	IV	V	VI	
Elymus trachycaulus	SSSSHHHH	0.85 (1-3)	17.84 (14-23)	0.65 (0-3)	4.38 (2-6)	-	-	Aung and Walton (1987)
Elymus canadensis#	SSSSHHHH	1.11 (1-4)	20.45 (14-27)	0.95 (0-3)	2.82 (0-4)	-	-	Present study
Elymus trachycaulus x *E. canadensis* (Amphiploids)	SSS'S'HHH'H'	1.50 (0-3)	24.30 (20-26)	0.20 (0-2)	2.50 (1-4)	-	-	Kumar and Walton (unpublished)
Elymus trachycaulus	SSSHHH	0.84 (0-4)	0.84 (0-4)	13.16 (10-14)	-	-	-	Aung and Walton (1987)
Elymus lanceolatus	SSSHHH	4.21 (0-9)	6.91 (0-13)	7.38 (3-14)	0.25 (0-2)	0.03 (0-1)	0.11 (0-1)	Sadasivaiah and Weijer (1981)
Elymus canadensis	SSSHHH	3.14 (1-8)	8.25 (0-16)	8.34 (0-9)	0.41 (0-3)	-	-	Present study

Values are mean for octoploids both regenerated and selfed.

Table 5.5. Mean and range of meiotic pairings in the metaphase I PMCs of the progenies of crosses, 8X x 4X and 6X x 4X, in *E. canadensis*

Cross	2n		chromosome associations				Nº of cells
			I	II	III	IV	
8X ECC x 4X ECC	42	Mean	2.55	16.89	2.21	0.10	71
		Range	0-9	9-20	0-6	0-1	
	40	Mean	3.52	14.02	2.52	0.04	96
		Range	1-8	5-19	0-9	0-1	
8X ECC x 4X ECM	43	Mean	4.26	15.26	2.59	0.11	46
		Range	1-9	6-20	0-10	0-1	
	42#	Mean	3.40	13.63	4.08	0.11	83
		Range	0-9	5-19	0-10	0-1	
	40#	Mean	4.28	16.83	2.05	0.10	58
		Range	0-14	9-19	0-5	0-1	
6X ECC x 4X ECC	35	Mean	2.29	15.01	0.89		76
		Range	0-5	11-17	0-4		
	30	Mean	1.11	13.32	0.8	0.04	108
		Range	0-4	10-15	0-3	0-1	
	29#	Mean	0.81	14.05	0.34	0.02	183
		Range	0-5	10-14	0-3	0-1	

Values are mean of two plants.

Table 5.6. Chromosome configurations of trisomic plants (2n=4x+1=29) of *E. canadensis* at metaphase I

Chromosome configuration	Plant 1		Plant 2	
	Nº of cells	%	Nº of cells	%
1IV + 11II + 3I	3	3.1	-	-
1IV + 1III + 11II	1	1.0	-	-
3III + 10II	1	1.0	-	-
2III + 11II + 1I	1	1.0	-	-
1III + 13II	36	37.5	17	19.5
1III + 12II + 2I	2	2.1	-	-
14II + 1I	52	54.2	62	71.3
13II + 3I	-	-	7	8
12II + 5I	-	-	1	1.1
Total	96	100.0	87	100.0

Fig. 5.1. Plant morphology of *E. canadensis* at different ploidy levels. **a.** white striped leaves of octoploid regenerant, **b.** octoploid regenerant, **c-e.** 3 month-grown plants, **c.** tetraploid (control), **d.** hexaploid, **e.** octoploid (selfed), **f-j.** 12 month-grown plants, **f.** tetraploid (control), **g.** hexaploid (backcrossed), **h.** pentaploid, **i.** tetrasomics, **j.** trisomics.

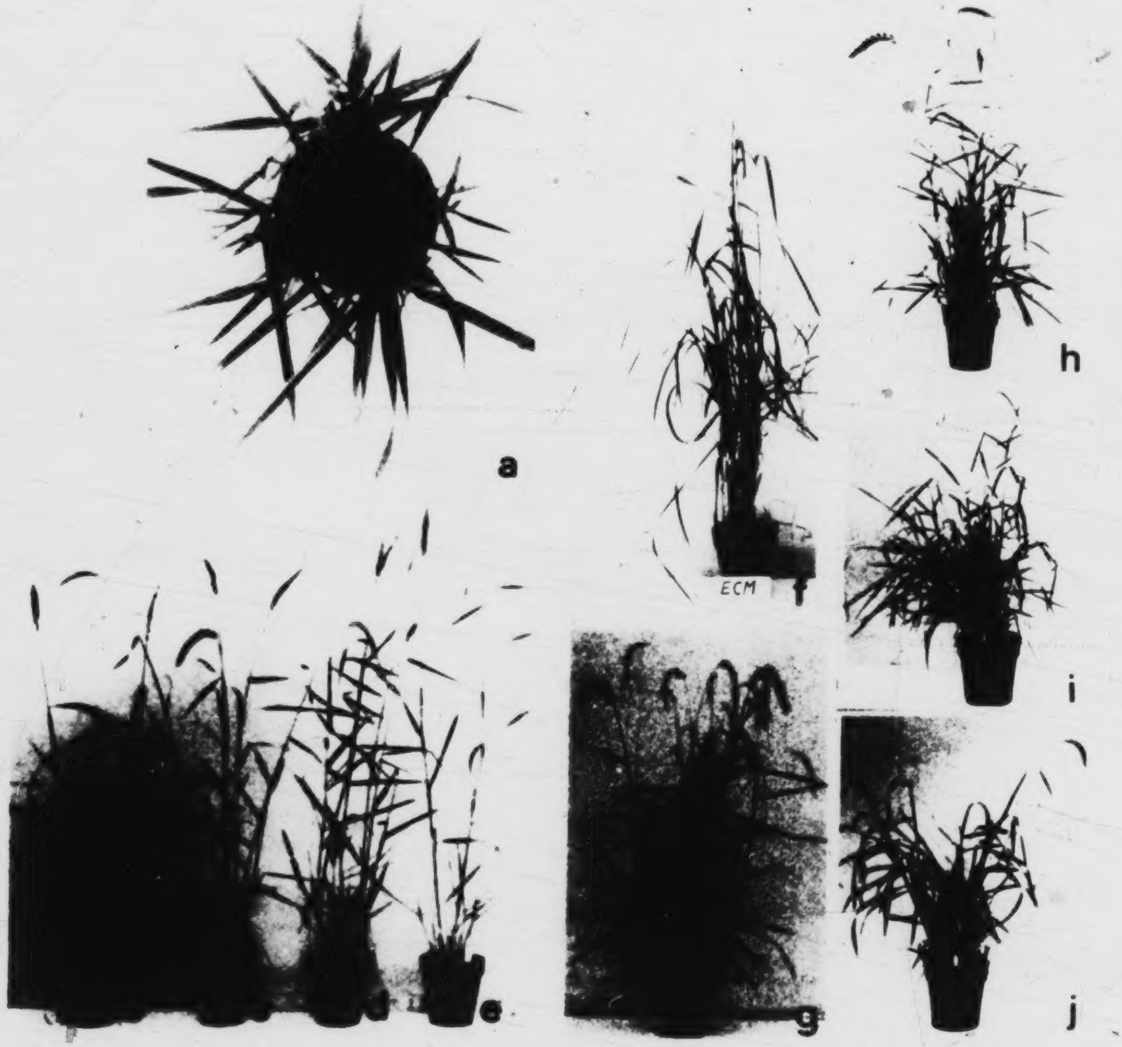

a
h
ECM
f
i
e
g
j

Fig. 5.2. Somatic chromosome complements of different ploidy levels of *E. canadensis*. **a.** octoploid ($2n=8x=56$), **b.** hexaploid ($2n=6x=42$), **c.** pentaploid ($2n=5x=35$), **d.** tetrasomics ($2n=4x+2=30$), **e.** trisomics ($2n=4x+1=29$).

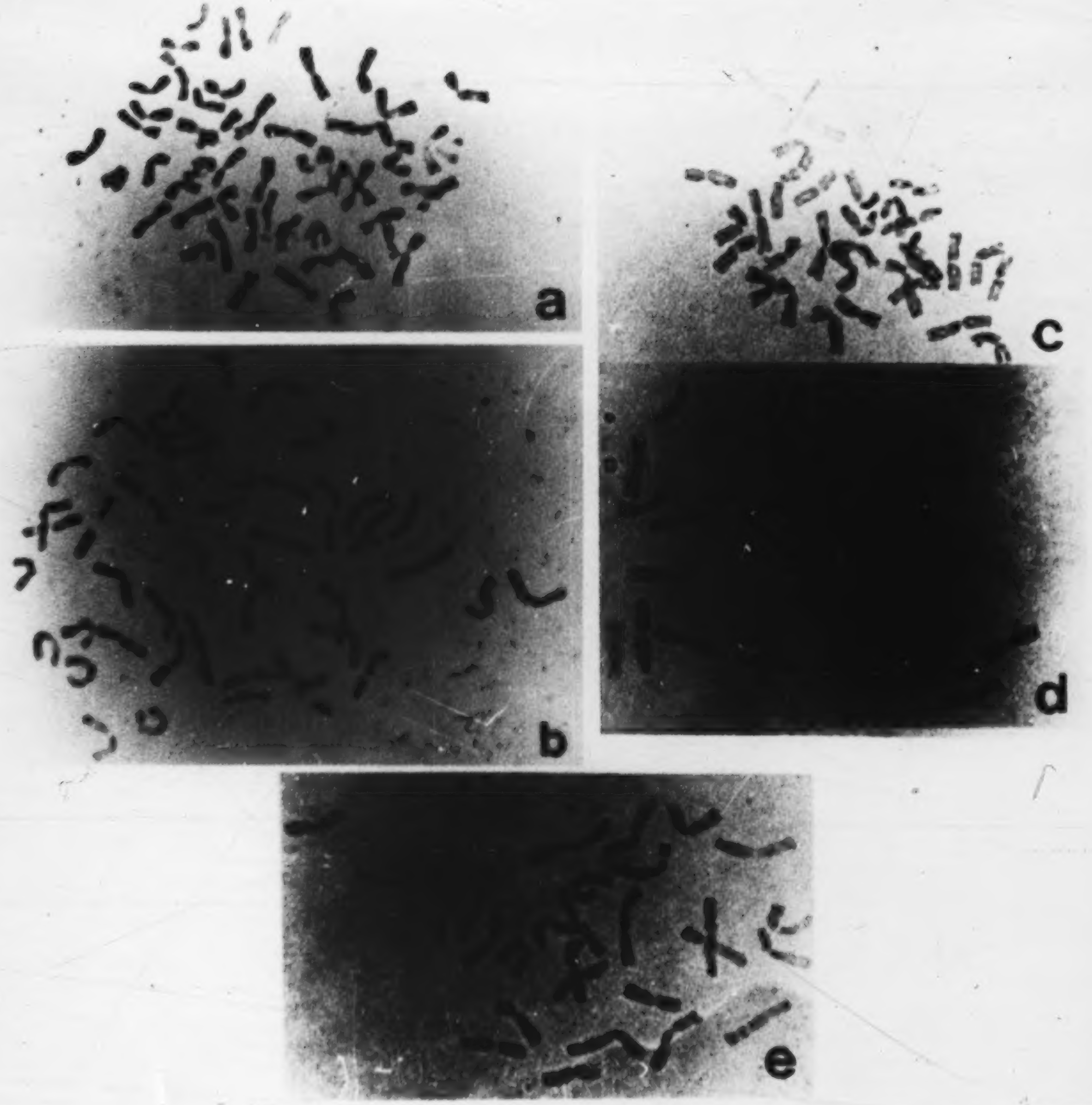

a
b
c
d
e

Fig. 5.3. Meiotic chromosome behavior of octoploid and hexaploid of *E. canadensis*. **a.** diakinesis of the octoploid (six quadrivalents), **b.** metaphase I of the octoploid (four quadrivalents), **c.** metaphase I of the octoploid (one quadrivalent), **d.** metaphase I of the hexaploid (nine trivalents), **e.** anaphase I of the hexaploid (seven laggards), **f.** anaphase II of the aneuploid (one laggard and chromatic bridge). Arrows indicate quadrivalents.

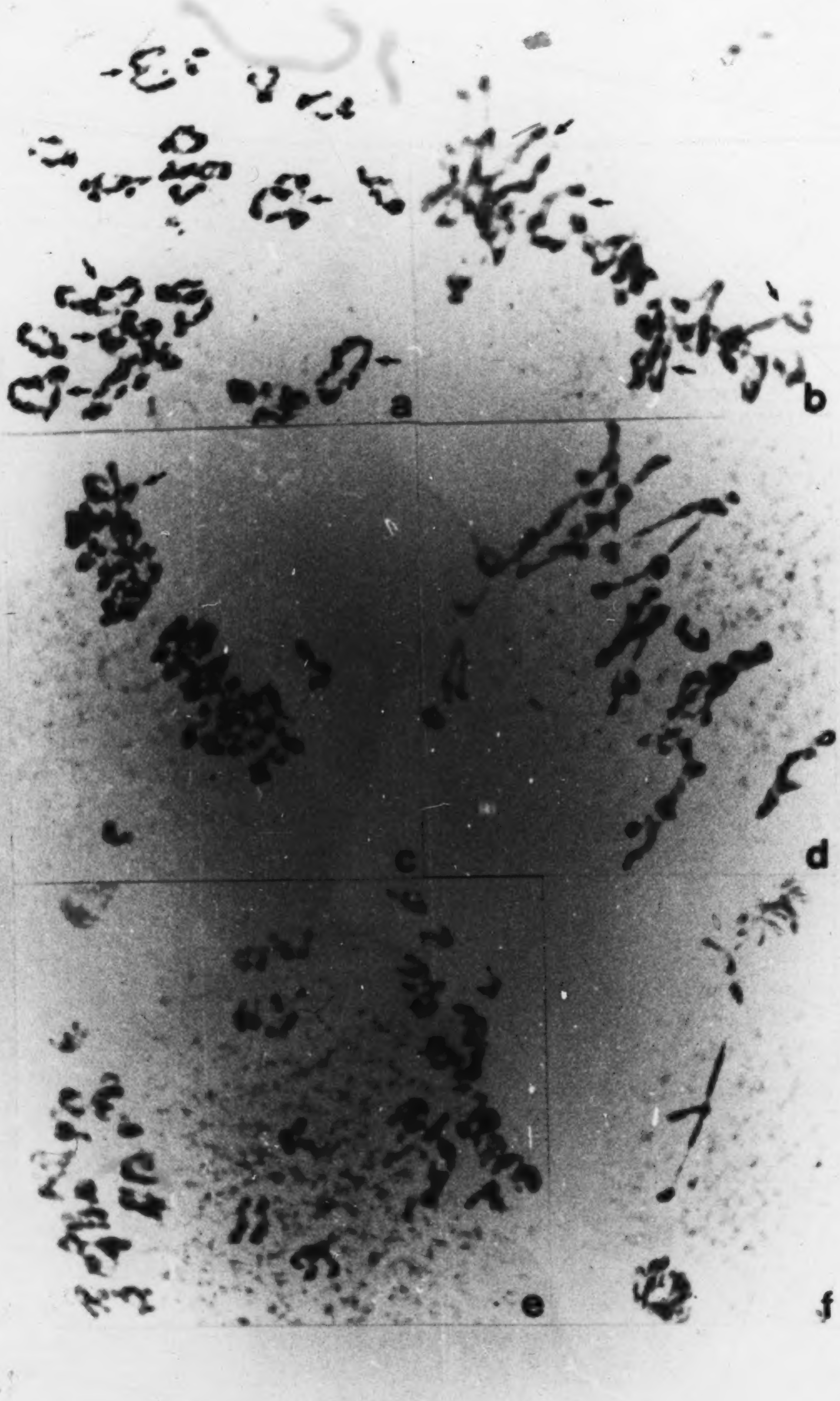
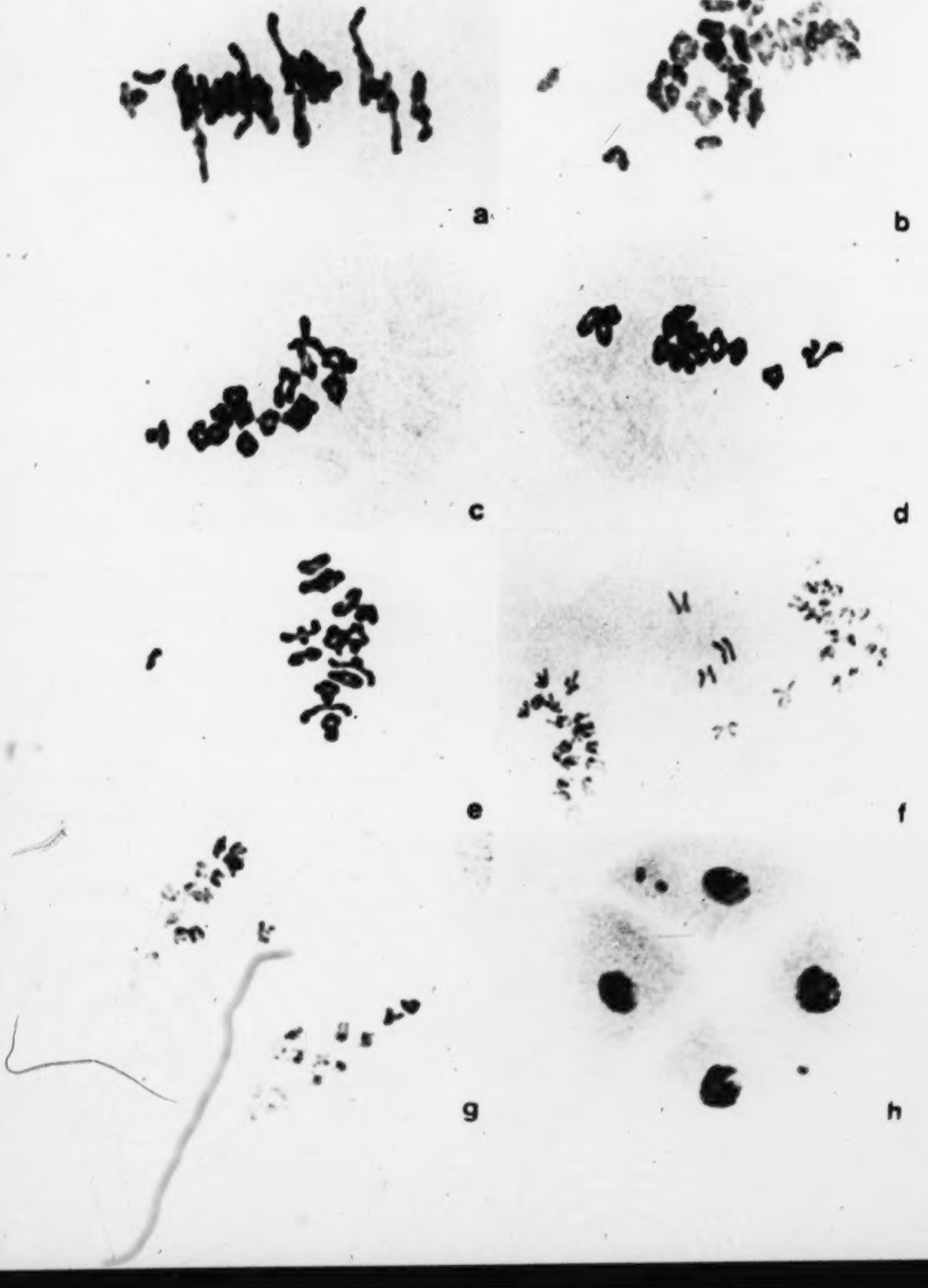

a
b
c
d
e
f

Fig. 5.4. Meiotic chromosome behavior of hexaploid (backcrossed), pentaploid, tetrasomics, and trisomics of *E. canadensis*. **a.** 1 I + 13 II + 5 III in a hexaploid PMC at MI, **b.** 3 I + 10 II + 4 III in a pentaploid PMC at MI, **c.** 1 I + 13 II + 1 III in a tetrasomic PMC at MI, **d.** 13 II + 1 III in a trisomic PMC at MI, **e.** 1 I + 14 II in a trisomic PMC at MI, **f.** five laggards in a hexaploid PMC at AI, **g.** one laggard in a trisomic PMC at AI, **h.** three micronuclei in a trisomic PMC at the tetrad stage.


a
b
c
d
e
f
g
h

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6. MORPHOLOGY, CYTOLOGY, AND FERTILITY OF TETRAPLOID PLANTS REGENERATED FROM AN IMMATURE INFLORESCENCE CULTURE OF *Elymus canadensis* [1]

6.1. Introduction

Many perennial forage grass cultivars have been developed by mass and recurrent selection of genetic variants in existing species (Walton 1983). Wide hybridization and polyploidy are also occasionally used as breeding strategies (Dewey 1984). In recent years, genetic variation and genomic rearrangement in tissue culture have been well documented for many crop species including the Gramineae (Lorz and Brown 1986; Larkin 1987).

Canada wildrye (*Elymus canadensis* L.) is a self-fertilized perennial grass native to U.S. and Canada which is sometimes grown as a cultivated species (Walton 1983). The species has been crossed with cereals (Mujeeb-kazi and Rodriguez 1982; Hang and Franckowiak, 1984; Mujeeb-kazi and Bernard 1985; Park and Walton 1989a) and forage grasses (Dewey 1984) to breed potentially disease resistant cereals or high yielding and good quality forages.

To examine the potential of tissue culture for the genetic variability of *E. canadensis*, the somatic tissues of immature embryos and immature inflorescences of *E. canadensis* were cultured and one

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1. A version of this chapter has been submitted for publication. Park & Walton 1989. J. Genetics & Breeding.

octoploid somaclone ($2n=8x=56$) and its selfed progeny were studied morphologically and cytologically (Park and Walton 1989b,c). The present study describes the variability in morphology, dry matter yield, chromosome behavior, and fertility among the somaclones with a normal tetraploid number of chromosomes ($2n=4x=28$).

6.2. Materials and Methods

6.2.1. Plant materials

A random sample of fifty six plants ($2n=4x=28$) from one hundred and seventy two somaclones which were obtained from immature inflorescence culture of *Elymus canadensis* (Park and Walton 1989b) was studied for morphological characters, dry matter yield, and fertility.

6.2.2. Morphological investigation

The characters investigated were plant height, stem height, number of tillers, leaf length, leaf width, spike length, dry weight, and leaf/stem ratio. The selected plants were grown in 5-inch pots in the greenhouse. Each plant was split into five clones of equal size to establish a randomized complete block trial with five replications. All characters were measured at maturity. Stem height was recorded as length from ground level to the top of stem, excluding the spike. Leaf sizes were measured at the longest and widest parts of the 2nd, 3rd, and 4th leaves from the top. The plants were cut at ground level and the vegetation was dried in an oven at 70 ^{o}C for two days and weighed.

The vegetatively propagated clones of a seed-sown Canada wildrye plant from which the explants for tissue culture were obtained were used as control. An analysis of variance for each character was computed by SASS.

6.2.3. Cytological observation

Eight somaclones, which differed substantially in their morphology from the parent plant, were used for meiotic study. All meiotic observations were made on pollen mother cells (PMCs) fixed in Carnoy's (6:3:1) solution and stained with 1.5% acetocarmine.

6.2.4. Fertility investigation

For all of the somaclones, seed fertility was recorded as the proportion of seeds set on open-pollinated spikes to the total number of florets. Pollen stainability was the percentage of pollen stainable with 2.0% acetocarmine solution. To study the nature of sterility, four completely sterile somaclones were emasculated and outcrossed with functional pollen of a control plant. The F_1 progeny were investigated for meiosis, seed fertility, and pollen stainability.

6.3. Results

6.3.1. Morphological characteristics and dry matter yield

Significant differences ($P<0.05$) were found between control and somaclones and within somaclones for dry matter yield and all the morphological characters except leaf length (Table 6.1). While all of these changes for quantitative traits are of interest, some are not of agronomic value because some traits such as leaf length, stem height, plant height, and spike length decreased (Fig. 6.1). In contrast, dry matter yield, tiller number, leaf width, and leaf/stem ratios of the somaclones showed values both higher and lower than the parent plant. Of the somaclones studied, 12 plants (21%) gave a higher dry matter yield than the parent plant from which they were derived and 15 plants (28%) gave a greater numbers of tillers than the parent. Leaves from fifteen somaclones were wider than those of the parent and 35 somaclones had a higher leaf/stem ratio. In Table 6.2, 10 high-yielding somaclones are listed together with their tiller numbers, mean leaf width, and leaf/stem ratio. I136-1 gave the highest yield (59% more than the control) followed by D100-3 and I135-1. Two genotypes had high tiller numbers, G112-1 and I136-1 being 112% and 67% higher, respectively, than the control. The leaf width for D100-3 was 35% wider than the control which was greater than for all other somaclones.

6.3.2. Chromosome behavior during meiosis

Eight somaclones used for cytological studies were selected based on their distinguishing gross morphological differences from the control (Fig. 6.2). Table 6.4 shows mean chromosome configurations at diakinesis and metaphase I, abnormalities of chromosome behavior at anaphase I and the tetrad stage, and the fertility of the control and eight somaclones. Changes in chromosome association at metaphase I were observed in the PMCs of all eight somaclones. Control plants showed predominantly well-connected bivalents (13.94 per cell) and infrequently unpaired univalents (0.12 per cell) at metaphase I (Fig.

6.3a). All somaclones occasionally formed multivalents at diakinesis (Fig. 6.3b) and showed chromosome associations including an increased number of univalents and a variable range of multivalents ranging from trivalent to hexavalent at metaphase I (Fig. 6.3c-e). The average number of bivalents among the somaclones was 12.01 to 13.57 per cell. Bivalents ranged from 2 to 14 per cell. E11-8 and H140-3 had an average of 1 univalent per cell and J200-3 had 2 univalents per cell. Isochromosomes were rarely observed at metaphase I. The frequencies of trivalents and quadrivalents were less than one per cell in all somaclones examined. Trivalents were mostly V-shape and quadrivalents tended to be chain-type. Pentavalents or hexavalents were rarely observed in D100-3, E11-8, and I144-1. Abnormalities of chromosome behavior at anaphase I and tetrad stage examined in this study included unequal distribution of chromosomes, lagging chromosomes, chromatid bridges, fragments, and micronuclei (Fig. 6.3f-h). In the control plant, the frequencies of the chromosome abnormalities were low: 2.2% in unequal distribution, 4.4% in laggards, 0% in bridges, and 2.6% in micronuclei. All somaclones increased significantly the frequencies of the different meiotic abnormalities, with on average 5.3% in unequal distribution, 9.0% in laggards, 3.9% in bridges, and 8.5% in micronuclei. In the somaclones, 2.6% (F8-2) to 7.4% (J200-3) of the total meiocytes at anaphase I, showed unequal distribution of chromosomes. The range of chromosome distribution in each pole was 11-17 (F8-2), 12-16 (D100-3, E11-8, I144-1, and J200-3), and 13-15 (D73-13, G112-1, and H140-3). From 5.7% (D73-13) to 13.5% (I144-1) of the meiocytes at anaphase I had from 1 to 7 laggards. From 0 (D73-13) to 5.9%

(D100-3) of the meiocytes had chromatid bridges at anaphase I. Chromosome fragments were uncommonly observed at anaphase I and II. In the tetrad stage, 3.3% (D73-13) to 12.8% (J200-3) of the meiocytes had from 1 to 4 micronuclei.

6.3.3. Seed fertility and pollen stainability

Seed fertility and pollen stainability averaged 53.8% and 58.5%, respectively, in the control plant. Seed fertility among the somaclones was variable, ranging from 0 to 45% (Fig. 6.1). Among 56 somaclones, 31 plants were completely sterile. Only three plants (C19-3, C19-4, and E11-10) had more than 20% seed fertility. Six plants had low rate of seed set, ranging from 16.4% to 10.2%. Seed fertility of 15 plants was less than 10%.

Pollen stainability of the somaclones differed among the clones (Fig. 6.4). The range of pollen stainability was from 56.1% in B26-2 to 1.5% in B28-1. Pollen stainability of 5 plants was as high as for the control plant. Twenty nine plants, comprising 52% of the total somaclones, exhibited pollen stainability between 24% and 49%. Five plants (B28-1, D100-1, E11-8, F82-3, and F82-5) had very low pollen stainability (1.5% to 6.2%).

To study the nature of sterility, four sterile somaclones (E11-8, G112-1, H140-3, and I144-1), for which meiosis was analyzed, were emasculated and pollinated with normal pollen of the control plant. Twenty two F_1 plants were obtained from this cross: 3 from E11-8, 6 from G112-1, 5 from H140-3, and 8 from I144-1. All of the F1 progeny behaved normally during meiosis. H140-3 had good seed fertility and pollen stainability in F_1. The F_1 progeny of the other three sterile somaclones pollinated with normal pollen were sterile and had variable pollen stainability, ranging from 1% to 32%.

6.4. Discussion

6.4.1. Morphological variation

For all morphological characters studied, the average values for the somaclones were lower than those for the parental seed-sown Canada wildrye plants. While average dry matter yield from the somaclones decreased; however, 10 genotypes gave higher yields and some had more tillers, a greater leaf width, and a higher leaf/stem ratio than the control plant. Some of the high yielding somaclones were dwarf and leafy with many tillers. These characteristics may make them satisfactory for grazing. Such materials are of potential breeding value provided the changes are heritable. However, sterility prohibited a study of inheritance.

Previously, somaclones obtained from embryogenic callus cultures of *Panicum maximum* (Hanna et al. 1984), *Pennisetum americanum* (Swedlund and Vasil 1985), *Zea mays* (Armstrong and Green 1985), and X *Triticosecale* Wittmack (Stolarz and Lorz 1986) were found to be relatively uniform in morphology. However, heritable genetic variation in morphology was found in fertile somaclones of cereal crops such as *Oryza sativa* (Sun et al. 1983), *Triticum aestivum* (Larkin et al. 1984; Maddock and Semple 1986; Chen et al. 1987), X *Triticosecale* Wittmack (Jordan and Larter 1985), *Zea mays* (Lee et al. 1988), and *Sorghum bicolor* (Bhaskaran et al. 1988). This study detected an obvious trend within the somaclone population for plants with reduced height and a more prolific tillering capacity relative to the parental plants.

6.4.2. Chromosomal variation

Chromosome structural changes were observed in all somaclones examined. At diakinesis and MI, multivalents were observed, indicating interchanges between nonhomologous chromosomes. An increased frequency of univalents probably resulted in the higher frequency of micronuclei in the tetrad stage. Observations of chromatid bridges and fragments indicate the occurrence of paracentric inversions. Unequal distribution of chromosomes at anaphase I was observed more frequently in somaclones than in the control plants and probably led to unbalanced gametes.

Chromosome rearrangements in tissue culture of the Gramineae have been observed in oats (McCoy et al. 1982), maize (Lee and Phillips 1987), tall fescue (Eizenga 1989), interspecific hybrids from crosses between *Hordeum vulgare* and *H. jubatum* (Orton 1980) and from crosses between *Lolium multiflorum* and *L. perenne* (Ahloowalia 1983), and intergeneric hybrids from crosses between*Triticum aestivum* and *Secale sereale* (Lapitan et al. 1984), and from between *Triticum crassum* and *Hordeum vulgare* (Fedak and Grainger 1986). The most frequent observations of chromosome structural changes in previous studies were heteromorphic pairs, univalents, multivalents, fragments, bridges, multiconstrictional chromosomes, and isochromosomes. These were indicative of deletions, duplications, and interchanges in which chromosome breakage and fusion were involved. The chromosome rearrangements observed in this study were apparently followed by genic alteration leading to phenotypic changes. Small changes in chromosome structure could alter expression and genetic transmission of specific genes, perhaps by deletion or duplication of one copy of a gene (Evans 1989).

6.4.3. Variation in fertility

Of all the characteristics studied, the greatest variability occurred in fertility. Meiotic instability is likely associated with low fertility or sterility of the somaclones. Reduction in fertility also occurred among the somaclones studied in wheat and triticale. The percentage of complete sterility ranged from 1.5% to 12.3% in wheat somaclones (SC_1 and SC_2) (Larkin et al. 1984) whereas the mean fertility of triticale somaclones was approximately 10% (SC_1) to 35% (SC_2) below that of parental plants (Jordan and Larter 1985).

In this study, seed set in the sterile somaclones pollinated with normal pollen indicates that sterility could be due to non-functional male gametes of the somaclones. Fertile progenies of one somaclone (H140-3) indicate that the sterility of H140-3 was due to meiotic irregularity, as shown in Table 6.4. However, three other sterile somaclones (E11-8, G112-1, and I144-1) still showed complete sterility in their outcrossed progeny in spite of normal meiosis. Therefore, the origin of sterility in the F_1 progeny of the three somaclones is not clear. With further studies of meiosis among the somaclone progeny may indicate the cause of sterility. Studies are also needed concerning the restoration of fertility over a few advanced generations by repeated pollination of sterile progeny with normal pollen. Male sterile variants showing heterosis in the progeny from a normal pollination were found in rice tissue culture but the nature of the male sterility has not been elucidated (Ling et al. 1987). Callus culture of cytoplasmic male sterile maize restored fertility (Earle et al. 1987). These studies indicate that tissue culture may induce sterility or restore fertility. The present study confirms reduction in fertility and complete sterility in tissue culture which is likely due to meiotic instability.

In conclusion, it was found in this study that *E. canadensis* in tissue culture is prone to changes in morpholoy, cytology, and fertility. However, results do not indicate conclusively whether the changes are genetic or epigenetic, because many of the somaclones did not give rise to selfed progeny at all, or else to insufficient numbers to determine the nature of the variability.

Table 6.1. Mean squares from analysis of variance for morphological characters of somaclones from *E. canadensis* and parental plants

Source of variance	df	Plant height	Stem height	Nº of tillers	Leaf length	Leaf width
Genotypes	56	617.26**	266.03**	864.21**	573.75NS	0.053**
control vs somaclones	1	185682.33**	77409.77**	198689.24**	8688.60**	14.390**
within somaclones	55	612.00**	265.74**	4837.41**	504.70NS	0.053**
Error	224	27.39	76.09	121.72	498.21	0.004

Source of variance	df	Spike length	Dry weight	Leaf/stem ratio	Pollen stainability	Seed fertility
Genotypes	56	9.48**	160.01**	1.14**	1117.91**	557.78**
control vs somaclones	1	5632.48**	6589.42**	27.28**	4826.69**	12123.10**
within somaclones	55	8.66**	160.93**	1.16**	1050.48**	347.49**
Error	224	2.38	17.94	0.26	27.82	3.08

F test significance: ** = P<0.01, NS = non-significance

Table 6.2. Some morphological characteristics for 10 high yielding somaclones evaluated in the greenhouse

Genotype	Dry weight (g/pot)	Tiller number	Leaf width (cm)	Leaf/stem ratio by dry weight
I136-1	28.6	51.4	0.89	1.05
D100-3	23.6	29.0	1.15	1.16
I135-1	22.7	40.6	0.93	1.18
F86-12	21.9	34.4	0.90	1.54
D73-13	21.4	29.0	0.81	1.44
E15-1	20.4	36.2	0.94	0.96
I135-3	20.2	34.4	0.82	1.49
G122-1	20.1	65.4	1.13	1.65
B26-2	19.5	42.0	0.82	1.28
C19-3	19.2	38.8	0.94	1.28
Control	18.0	30.8	0.85	1.16
SD#	2.9	10.9	0.12	0.22

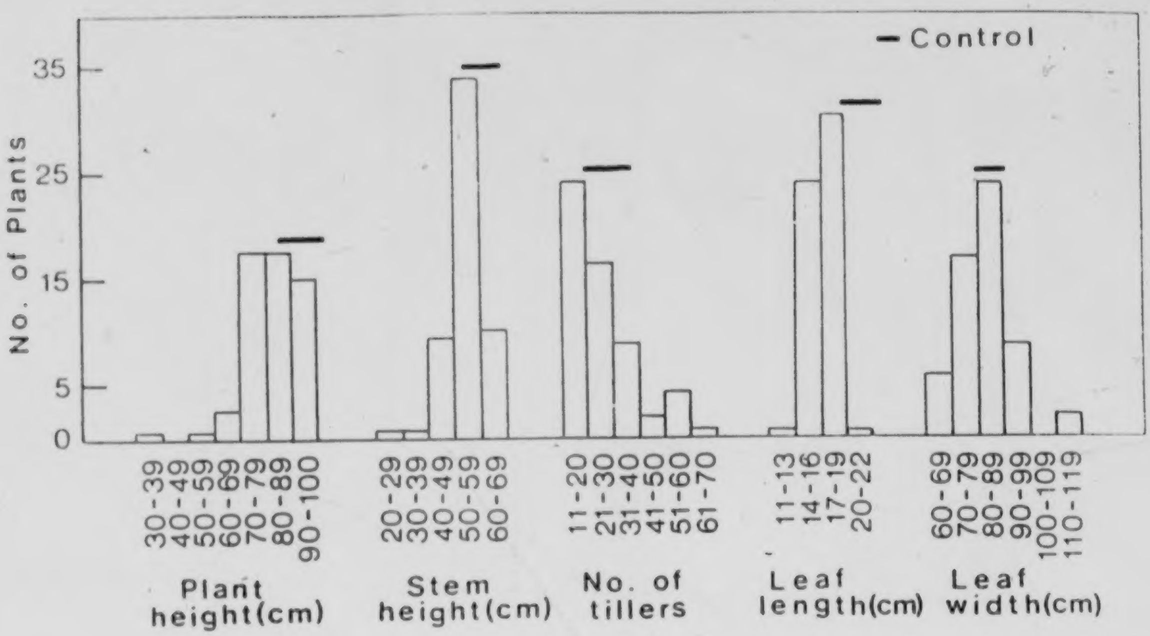
SD = Standard Deviation

Table 6.3. Chromosome associations and abnormalities at meiosis and fertility of control and eight somaclones of *E. canadensis*

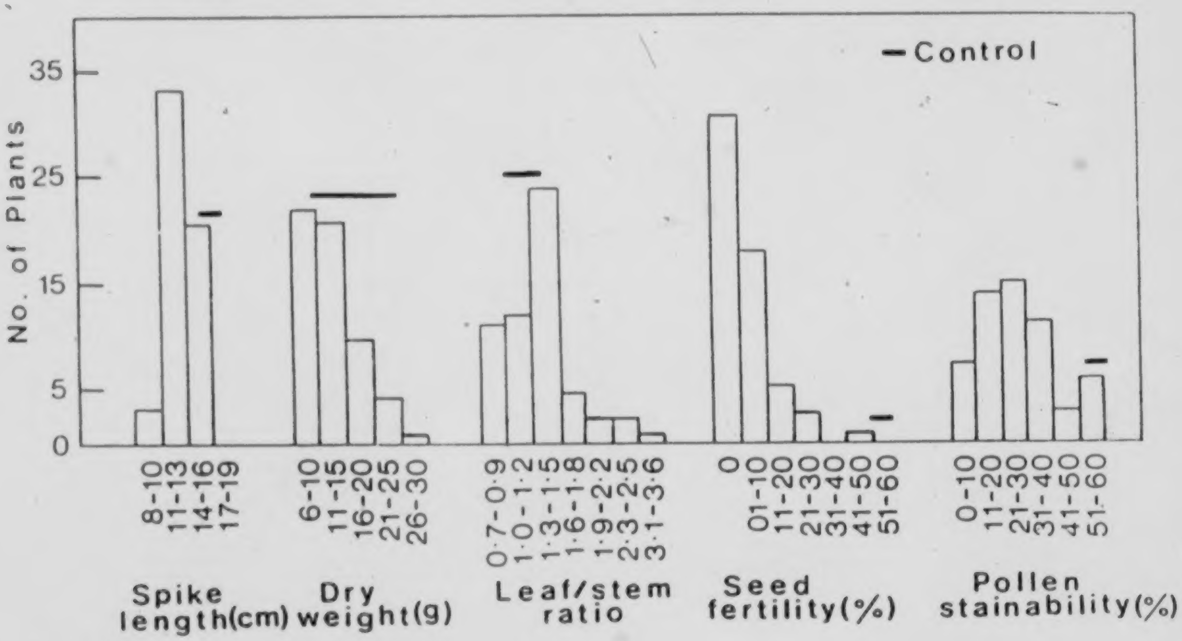
Genotype	#Nº of multivalents at DIK	Mean chromosome associations (Range) I	II	III	IV	V	VI	Nº of cells	Nº of abnormalities/cell (Range) Unequal distribution	Lagging chromosome	Chromatid bridge	Micronuclei	Fertility Pollen (%)	Seed (%)
Control	0	0.12 (0-4)	13.94 (12-14)					49	0.02	0.04 (0-2)	0	0.03 (0-1)	58.5	53.8
D73-13	0.22 (0-1)	0.87 (0-8)	12.17 (6-14)	0.09 (0-1)	0.17 (0-2)			46	0.04	0.06 (0-4)	0	0.03 (0-1)	31.7	2.1
D100-3	0.76 (0-2)	0.21 (0-3)	12.06 (2-14)	0.17 (0-4)	0.36 (0-3)	0.01 (0-1)	0.01 (0-1)	70	0.05	0.09 (0-3)	0.06 (0-1)	0.07 (0-3)	42.1	5.5
E11-8	0.47 (0-2)	1.17 (0-4)	12.62 (4-14)	0.2 (0-2)	0.15 (0-2)	0.01 (0-1)		40	0.06	0.1 (0-3)	0.03 (0-1)	0.11 (0-2)	2.6	0.0
F8-2	0.15 (0-1)	0.47 (0-4)	13.57 (9-14)	0.06 (0-2)	0.05 (0-1)			77	0.03	0.08 (0-6)	0.03 (0-1)	0.03 (0-1)	16.4	1.5
G112-1	0.25 (0-1)	0.84 (0-4)	13.15 (8-14)	0.12 (0-1)	0.09 (0-1)			48	0.06	0.07 (0-4)	0.05 (0-2)	0.11 (0-3)	14.6	0.0
H140-3	0.38 (0-2)	1.02 (0-17)	13.10 (2-14)	0.18 (0-1)	0.24 (0-1)			69	0.07	0.08 (0-3)	0.04 (0-1)	0.09 (0-3)	35.3	0.0
I144-1	0.34 (0-2)	0.9 (0-3)	12.01 (9-14)	0.11 (0-1)	0.27 (0-2)		0.01 (0-1)	52	0.04	0.14 (0-3)	0.05 (0-1)	0.08 (0-2)	17.3	0.0
J200-3	0.42 (0-1)	1.91 (0-7)	12.42 (6-14)	0.23 (0-1)	0.16 (0-2)			43	0.07	0.11 (0-7)	0.04 (0-1)	0.13 (0-2)	28.6	1.1
Mean(Total) of somaclones	0.37	0.92	12.64	0.15	0.19	0	0	(445)	0.05	0.09	0.04	0.08	23.6	1.3

Nº of multivalents/cell at diakinesis

Fig. 6.1. Frequency distributions of Canada wildrye somaclones and range of control (bold bar) for morphological characters, dry matter yield, and fertility.



— Control
No. of Plants
35
25
15
5
0
30-39
40-49
50-59
60-69
70-79
80-89
90-100
Plant height(cm)
20-29
30-39
40-49
50-59
60-69
Stem height(cm)
11-20
21-30
31-40
41-50
51-60
61-70
No. of tillers
11-13
14-16
17-19
20-22
Leaf length(cm)
60-69
70-79
80-89
90-99
100-109
110-119
Leaf width(cm)



— Control
No. of Plants
35
25
15
5
0
8-10
11-13
14-16
17-19
Spike length(cm)
6-10
11-15
16-20
21-25
26-30
Dry weight(g)
0·7-0·9
1·0-1·2
1·3-1·5
1·6-1·8
1·9-2·2
2·3-2·5
3·1-3·6
Leaf/stem ratio
0
01-10
11-20
21-30
31-40
41-50
51-60
Seed fertility(%)
0-10
11-20
21-30
31-40
41-50
51-60
Pollen stainability(%)

Fig. 6.2. Plant morphology of Canada wildrye somaclones selected for meiotic analysis. **a.** semidwarf, long leaves, sterility, **b.** semidwarf, short and broad leaves, low fertility, **c.** semidwarf, low number of tillers, sterility, **d.** semidwarf, thick stems, short and broad leaves, low fertility, **e.** dwarf, compact, short and broad leaves, sterility, **f.** dwarf, narrow leaves, low fertility, **g.** dwarf, compact, short and broad leaves, sterility, **h.** dwarf, narrow leaves, high number of tillers, leafy. low fertility.

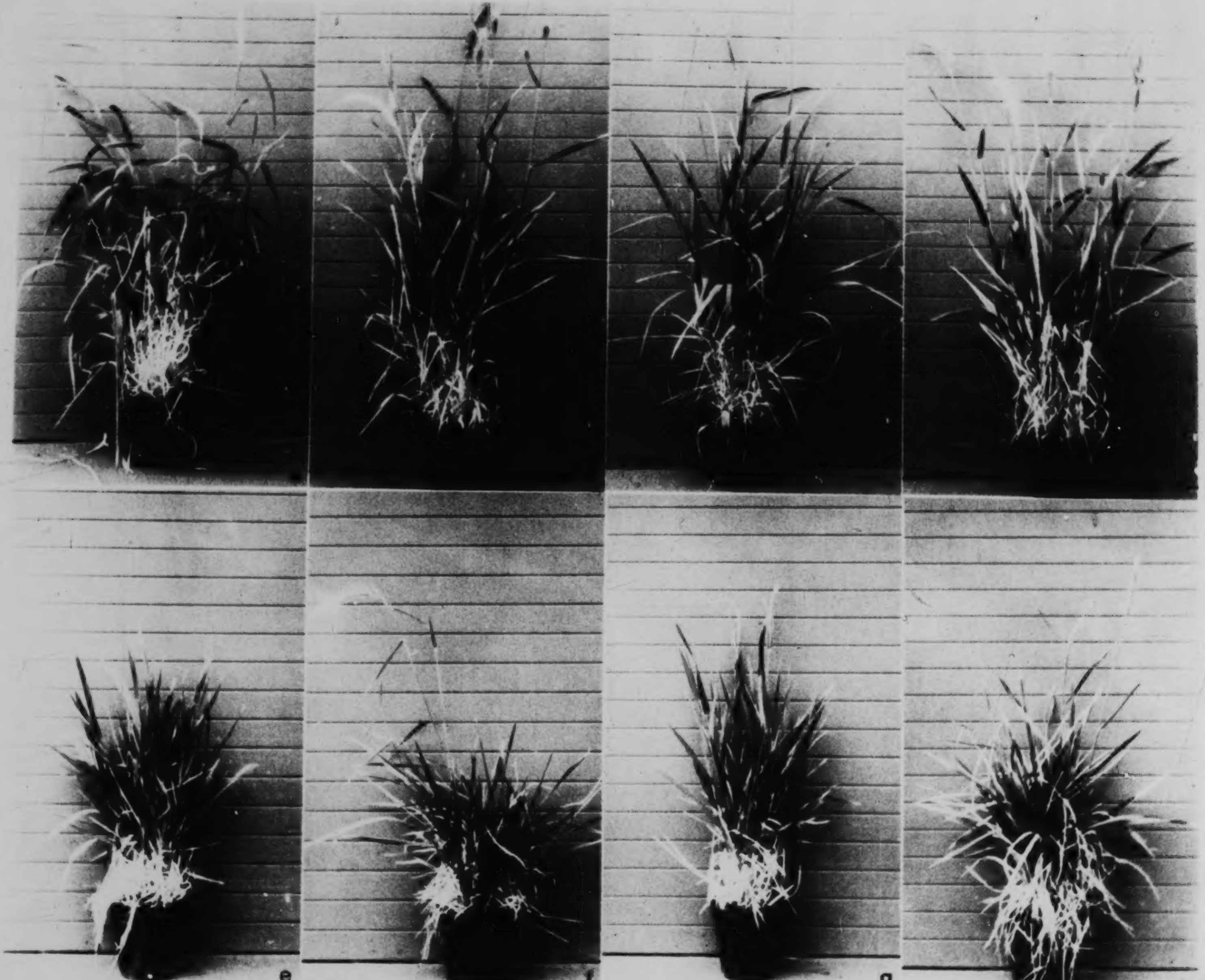

e
f
g

Fig. 6.3. Meiotic chromosome behavior of eight somaclones of *E. canadensis*. **a.** fourteen bivalents in a control plant PMC at MI, **b-h.** meiotic irregularity in somaclone PMCs,.**b.** hexavalent (arrow) and quadrivalent association at diakinesis, **c.** one quadrivalent at MI, **d.** two quadrivalents (long arrows), one fan type trivalent (short arrow), and one isochromosome (dark triangle) at MI, **e.** one V-shape trivalent (arrow), four bivalents, and seventeen univalents at MI, **f.** two lagging chromosomes at AI, **g.** chromatid bridge (long arrow) and fragment (short arrows) at AI, **h.** micronuclei at the tetrad stage.

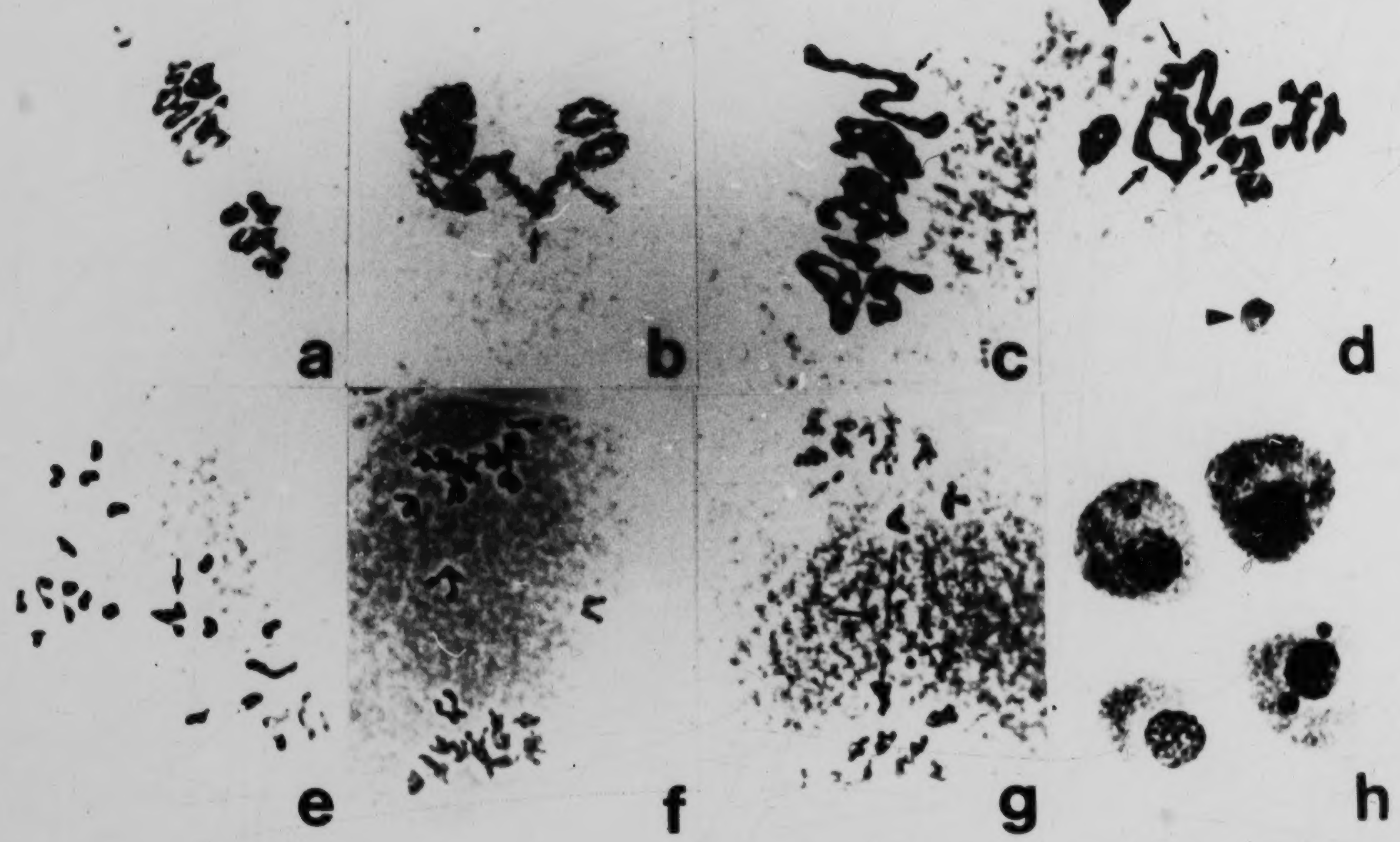
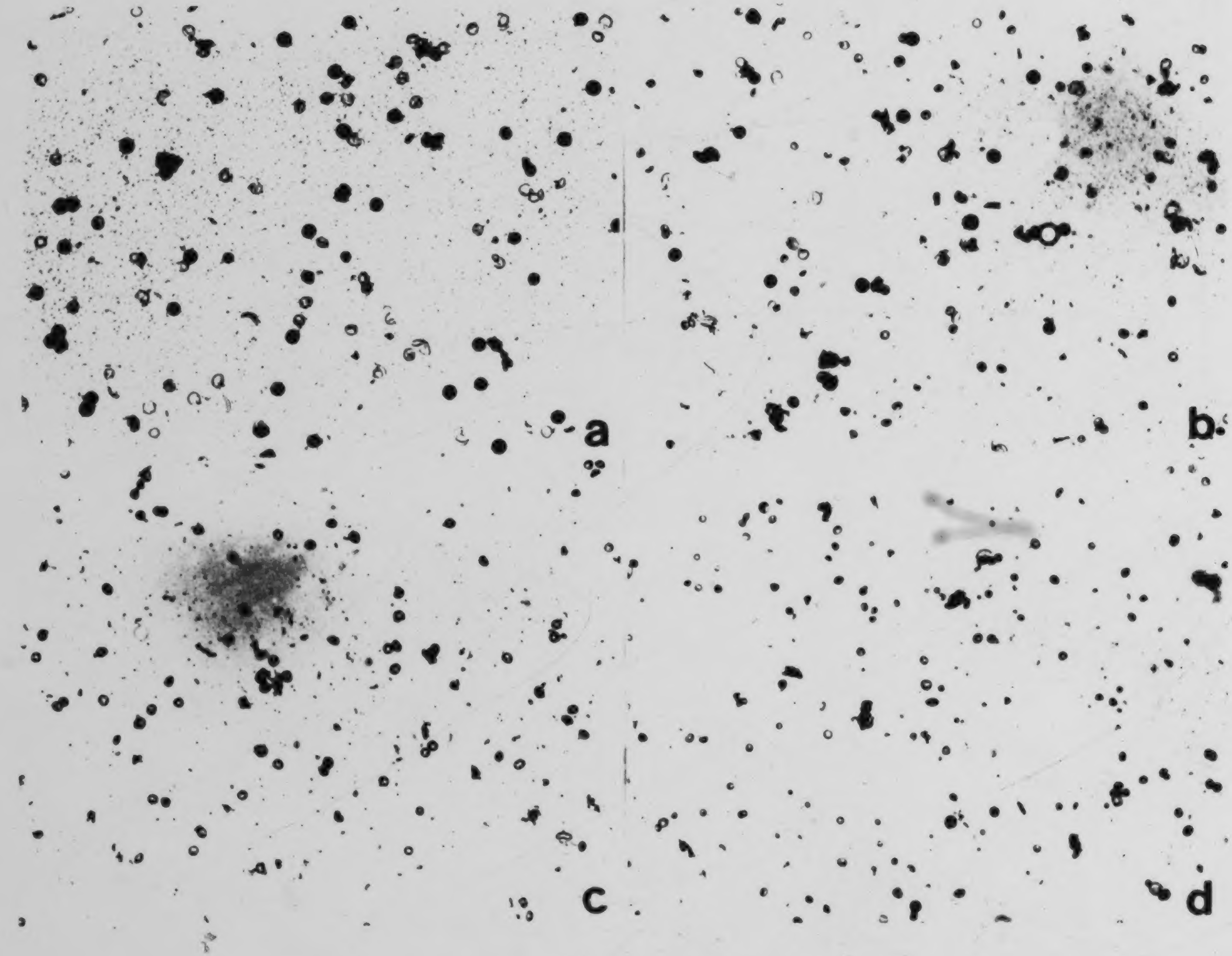

a
b
c
d
e
f
g
h

Fig. 6.4. Pollen stainability in the somaclones of *E. canadensis*.

a. Control, **b.** J200-3, **c.** E11-8, **d.** G112-1


a
b
c
d

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7. DISCUSSION AND CONCLUSIONS

In this study, creation of autopolyploids and allopolyploids of *Elymus canadensis* has been attempted by intergeneric hybridization, somatic tissue culture, and interploidy hybridization. The plants developed in this study include triploids (SHN and SHR), tetraploids (SHRR), pentaploids (SSHHH or SSSHH), hexaploids (SSHHNN, SSHHRR, and SSSHHH), octoploids (SSSSHHHH), and some other aneuploids including tetrasomics and trisomics of *E. canadensis*.

Allotriploids of the same genome constitution were previously produced between *Elymus scribneri* (SSHH) and *Psathyrostachys juncea* (NN) by Dewey (1967a) and between *Elymus canadensis* (SSHH) and *Secale cereale* (RR) by Hang and Franckowiak (1984). However, the allohexaploids (SSHHNN and SSHHRR) are reported for the first time in this study. Genomic disharmony and meiotic instability in these allohexaploids led to undesirable phenotypes and sterility. In terms of potential breeding value, the serious instability of the allohexaploids even in early generations is in contrast to what occurs in the man-made allohexaploid triticale, which showed genetic and cytogenetic instability in relatively late generations (Muntzing 1979).

Chromosome behavior of these allohexaploids was comparable to that of the segmental autoallohexaploids (SSS'S'HH) between the species with a common genome (Dewey 1974, Asay and Dewey 1976). Chromosome pairing of these allohexaploids (SSHHNN and SSHHRR) was also comparable to that in wheat x rye hexaploid hybrids (AABBRR). The *ph* system on wheat chromosome 5B suppressing homoeologous pairing has been shown to affect the pairing of rye chromosomes in both octoploid and hexaploid triticale (Riley and Miller 1970; Thomas and Kaltsikes 1971). The genetic regulation of diploid-like meiotic behaviour has been known in many segmental allopolyploids including *Triticum aestivum* (Riley and Chapman 1958), *Avena sativa* (Rajhathy and Thomas 1972), and *Festuca arundinacea* (Jauhar 1975). Predominately intragenomic pairing in these allohexaploids resulting from nonhomology among the parental genomes indicates that they are genomic allohexaploids without genetic control of meiotic pairing. In fact, the female parent of the allohexaploids, *E. canadensis* is a strict allotetraploid (Dewey 1966, 1967b). Autosyndetic pairing in SSHH genomes of *E. canadensis* might be attributed to natural hybridization and spontaneous doubling of the chromosome numbers of the F_1 hybrids between diploid progenitors (SS and HH species). On the basis of multivalent patterns, this study indicates that there is some capability of randomly homoeologous pairing (S-H, H-N, or S-N in *E. canadensis* x *P. juncea* hybrids and S-H, H-R, or S-R in *E. canadensis* x *S. cereale* hybrids). Where extensive intergenomic pairing has taken place, alien addition or substitution lines carrying desirable genes from the alien species could be obtained from backcrosses of these allohexaploids to the parents. However, as discussed earlier, cross incompatibility makes it difficult to produce backcross progeny in both allohexaploids with the exception of the backcross of SSHHRR to RR. Accordingly, overcoming the cross incompatibility barrier would be a prerequisite to the enhancement of introgression for developing new germplasm associations.

This study also morphologically and cytologically characterized a tissue culture-derived octoploid of *E. canadensis* and its selfed and backcrossed progenies. The octoploid of *E. canadensis*, [genome formula (SSSSHHHH)], is not found in nature (Bowden 1964). Predominance of bivalents even in the octoploids indicates a possible diploidizing system in these autoallopolyploids, as suggested by Kumar and Walton (unpublished). This autoalloploid exhibited phenotypically general features of autopolyploidy, i. e. reduction in tiller number and fertility, and gigas leaf and stem sizes. However, in terms of forage production, reduced biomass yield made these octoploids unsuitable for practical agronomic gains. Although the breeding behavior of the octoploid *E. canadensis* was not studied systematically, a high proportion of octoploid progeny in the S_1 population suggests that the maintenance of the octoploid in advanced generations may be possible.

A natural hexaploid *E. lanceolatus* with the genome formula of SSSHHH resulted from the fertilization between unreduced gametes and normally reduced gametes (Sadasivaiah and Weijer 1981). This hexaploid *E. lanceolatus*, which exists in nature, indicates that the double triploid type of *Elymus* species (SSSHHH) is not beyond the optimum ploidy level. However, the induced SSSHHH hexaploids in the present study were cytologically unstable, leading to a reduction in fertility. In the following selfed generation, most of the offspring were aneuploids with variable numbers of chromosomes (Park, unpublished). In the subsequent generations of the hexaploid *E. trachycaulus*, increase of chromosome instability is assumed to be responsible for drastic decreases in morphological vigor and forage yield (Walton, unpublished). Consequently, the polyploids induced in this study are not competitive with the natural tetraploid *E. canadensis* in relation to forage yield and genetic or cytogenetic stability. These results are consistent with Dewey's skepticism of the induction of polyploidy at higher ploidy levels than hexaploid (Dewey 1979).

Induced polyploids from this study can be used as a bridging species for wide crossses, and the aneuploid stocks can be used for genetic studies. The octoploid *E. canadensis* may be hybridized with annual or perennial diploid Triticeae species with higher cross compatibility compared to the tetraploid *E. canadensis*. In fact, one S_1 octoploid plant has been hybridized with the diploid *Agropyron cristatum* cv, Parkway and produced a pentaploid F_1 hybrid (Park, unpublished). A fertile pentaploid hybrid was obtained between the octoploid *Elymus trachycaulus* and the diploid *Pseudoroegneria spicata* (Aung and Walton 1987), and has been backcrossed to *P. spicata* to introduce drought tolerance (Walton, unpublished).

Primary aneuploid stocks are useful for genetic studies and for an understanding of genome nature (Khush 1973). Tetrasomics and trisomics of *E. canadensis* are reported for the first time in this study. Further extensive 6X x 4X crosses, and selfing of the fertile tetrasomics and trisomics, would increase the number of trisomics with potential for producing a complete trisomic series of *E. canadensis* . In this trisomic series, the nature of the S and H genome in *E. canadensis* could be precisely determined if each extra chromosome and a marker gene were identified.

In addition, this study indicates a possible application of somatic tissue culture to genetic or epigenetic modification in this species.

Embryo-derived callus formation and plant regeneration has not previously been employed for the production of the SHN and SHR hybrids. The health and maturity of the SHR hybrid plants obtained through such methods may be indicative of tissue culture-induced chromosome rearrangements followed by genic or epigenetic modification, to suppress gene expression that might otherwise result in hybrid necrosis. Besides multivalent formation, other indications of chromosome rearrangements in the SSHHRR amphiploids were the ability to backcross the amphiploid to the male parent, *S. cereale*. SSHHNN amphiploids obtained through the conventional embryo culture failed to backcross to either of the parents. Fedak and Grainger (1986) indicated a potential application of callus culture to cause epigenetic modifications that would lead to a change in incompatibility barriers. The potential to backcross regenerants was greater than for the original intergeneric sterile hybrids.

Changes in chromosome structure were detected among the regenerants of *E. canadensis*. Although some of the somaclones were more productive, their chromosomal instability led to complete sterility or low fertility. Accordingly, it is not known whether the somaclonal variation in morphology, cytology, and fertility is heritable or epigenetic. Further studies are needed with large populations of fertile somaclones to determine the inheritance of somaclonal variation of this species. For genome manipulation, immature inflorescence culture was effective for this native grass species.

From the information discussed above, some general conclusions can be made about the genome manipulation of *E. canadensis* through intergeneric hybridization, somatic tissue culture, and interploidy hybridization for the purpose of developing more adaptive germplasms as forage grasses.

1. Genomic allohexaploids of *E. canadensis* with two alien species, *P. juncea* and *S. cereale*, that do not carry a common genome is not a promising procedure to develop improved forage grass germplasm.
2. Genomic disharmony and chromosomal instability in these intergeneric hybrids limit gene transfer of desirable character from either of the parents.
3. Plant regeneration from hybrid embryo-derived callus culture is apparently an alternative to rescue of undifferentiated hybrid embryos and apparently overcomes hybrid necrosis.
4. Genome manipulation and cytological variability of *E. canadensis* can occur by changing chromosome number and structure using somatic tissue culture. However, somaclonal variants have limited direct value for forage production, but may contribute to further genetic studies of the species.
5. It is not recommended to autoallopolyploidize *E. canadensis* for use as a forage grass. However, hexaploids of the double triploid type are worthy of further study, to gain agronomic potential and to understand the nature of the genomes.

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APPENDIX

Genomically based nomenclature and traditional nomenclature of perennial Triticeae species referred to in this study

Genus	Species	Taditional nomenclature
Agropyron	*Agropyron cristatum*	*Agropyron cristatum*
	Agropyron desertorum	*Agropyron desertorum*
Pseudoroegneria	*Pseudoroegneria libanotica*	*Agropyron libanoticum*
	Pseudorogneria spicata	*Agropyron spicatum*
Psathyrostachys	*Psathyrostachys juncea*	*Elymus junceus*
Critesion	*Critesion bogdanii*	*Hordeum bogdanii*
	Critesion californicum	*Hordeum californicum*
Thinopyrum	*Thinopyrum curvifolium*	*Agropyron curvifolium*
Elytrigia	*Elytrigia repens*	*Agropyron repens*
Elymus	*Elymus canadensis*	*Elymus canadensis*
	Elymus caninus	*Agropyron caninum*
	Elymus ciliaris	*Agropyron ciliare*
	Elymus drobovii	*Agropyron drobovii*
	Elymus elymoides	*Elymus sitanion*
	Elymus glaucus	*Elymus glaucus*
	Elymus lanceolatus	*Agropyron dasystachyum*
	Elymus mutabilis	*Agropyron mutabilis*
	Elymus patagonicus	*Elymus patagonicus*
	Elymus scribneri	*Agropyron scribneri*
	Elymus sibiricus	*Elymus sibiricus*
	Elymus tilcarensis	*Agropyron tilcarense*
	Elymus trachycaulus	*Agropyron trachycaulum*
	Elymus trachycalus spp. subsecundus	*Agropyron subsecundum*
	Elymus tsukushiensis	*Agropyron tsukushiense*
	Elymus yezoensis	*Agropyron yezoense*
Leymus	*Leymus cinereus*	*Elymus cinereus*
	Leymus innovatus	*Elymus innovatus*
	Leymus triticoides	*Elymus triticoides*
	Leymus secalinus	*Elymus secalinus*